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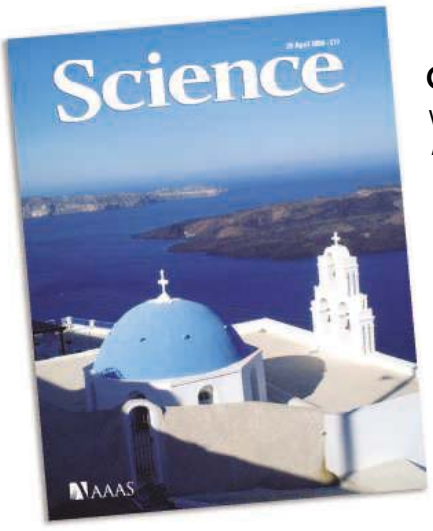


## COVER

View of Santorini, Greece, from Fira. Across the volcanic caldera, the Nea and Palea Kameni islands (middle right) have been volcanically active since 197 B.C. The Akrotiri peninsula (top left) was an area of major Bronze Age settlement that was destroyed but preserved by the Minoan eruption in the late 17th century B.C. See pages 548 and 565.

***Photo: Sturt Manning***

## DEPARTMENTS



## EDITORIAL



## NEWS OF THE WEEK



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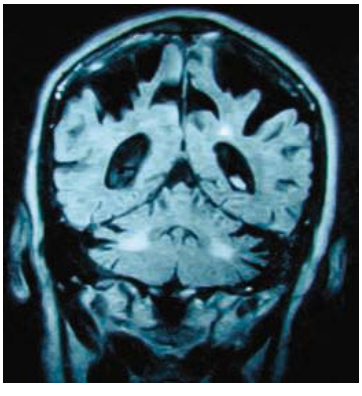

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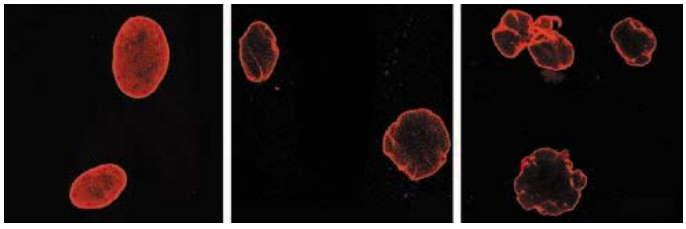

## SCIENCE EXPRESS

www.sciencexpress.org

DEVELOPMENTAL BIOLOGY

**Wnt Gradient Formation Requires Retromer Function in Wnt-Producing Cells**

*D. Y. M. Coudreuse, G. Roël, M. C. Betist, O. Destrée, H. C. Korswagen*

A multiprotein complex that transports molecules into cells is required for formation of a protein gradient that patterns developing tissues in animals.

10.1126/science.1124856

CELL BIOLOGY

**CRACM1 Is a Plasma Membrane Protein Essential for Store-Operated $Ca^{2+}$ Entry**

*M. Vig* et al.

Two membrane proteins, which control calcium flow into cells upon depletion of intracellular calcium stores, are either part of the elusive calcium release–activated calcium channel or act as its regulators.

10.1126/science.1127883

CELL BIOLOGY

**Lamin A–Dependent Nuclear Defects in Human Aging**

*P. Scaffidi and T. Misteli*

Sporadic defects in the lamin A protein, which helps form the architecture of the nucleus, have been implicated in a premature aging disease and are also responsible for normal aging.

10.1126/science.1127168

MICROBIOLOGY

**Emergent Properties of Reduced-Genome *Escherichia coli***

*G. Pósfai* et al.

Targeted deletions of up to 15 percent of the genome of a common bacterium yielded new and improved strains, including ones that could take up foreign DNA more efficiently.

10.1126/science.1126439

## TECHNICAL COMMENT ABSTRACTS

CLIMATE CHANGE



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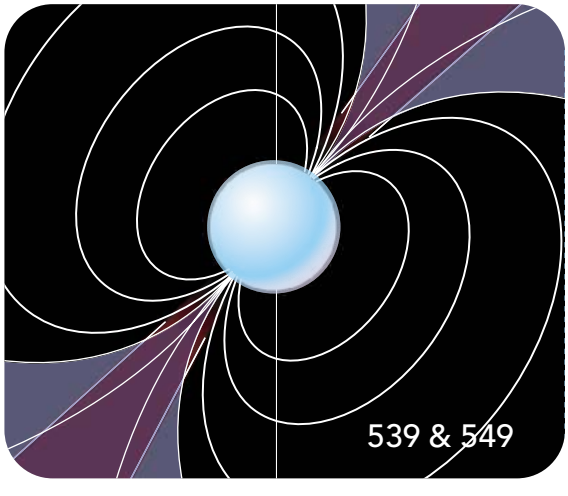


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APPLIED PHYSICS



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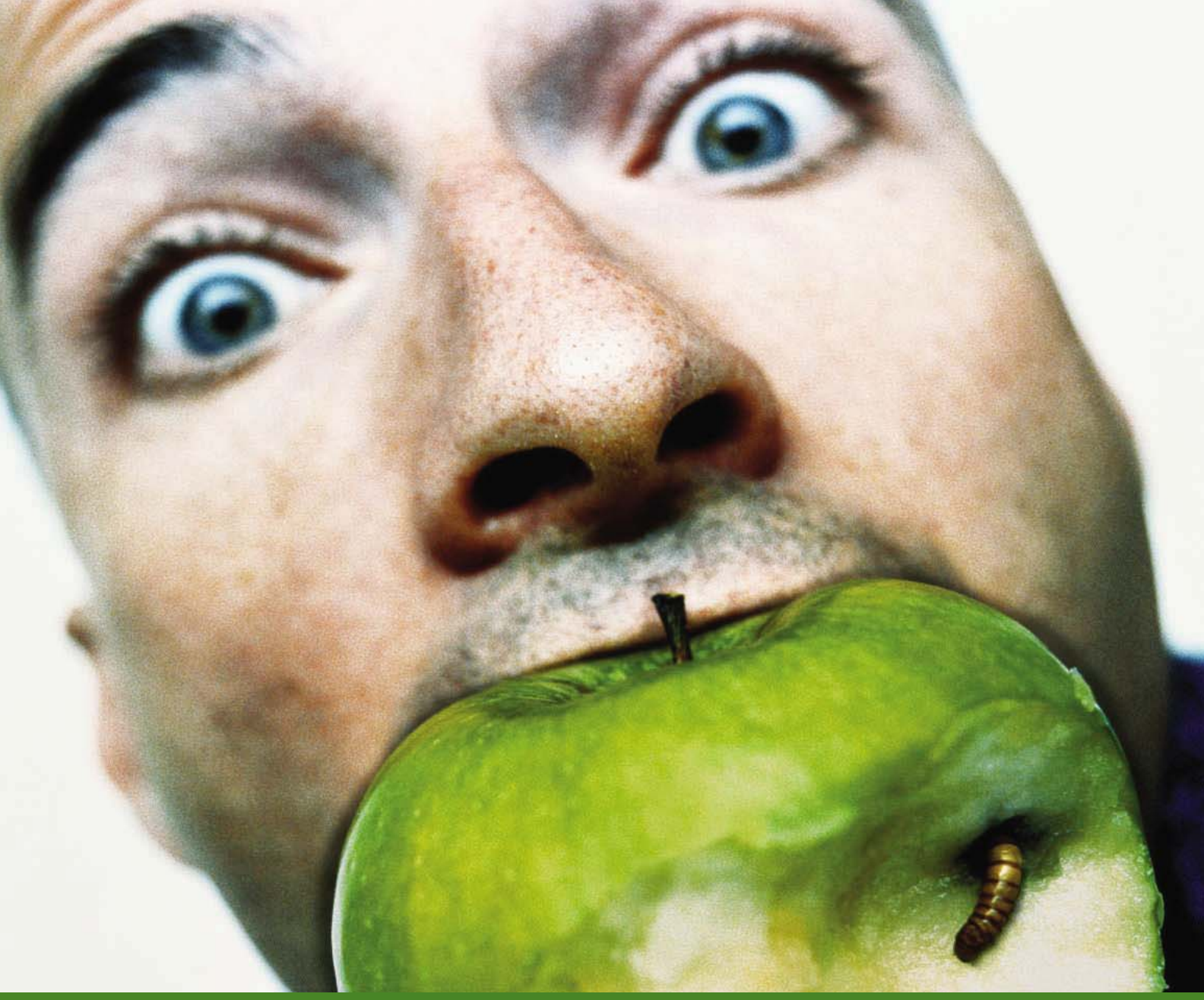
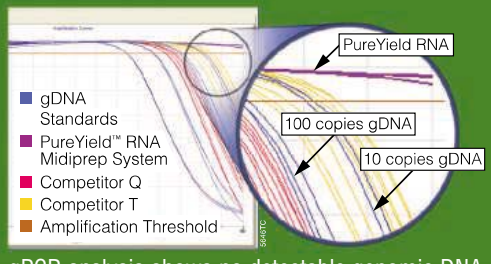


# Is unexpected DNA worming its way into your RNA?

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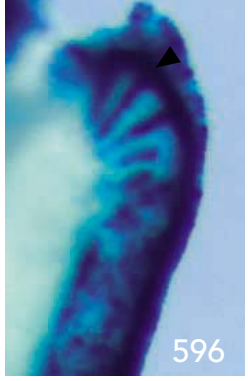




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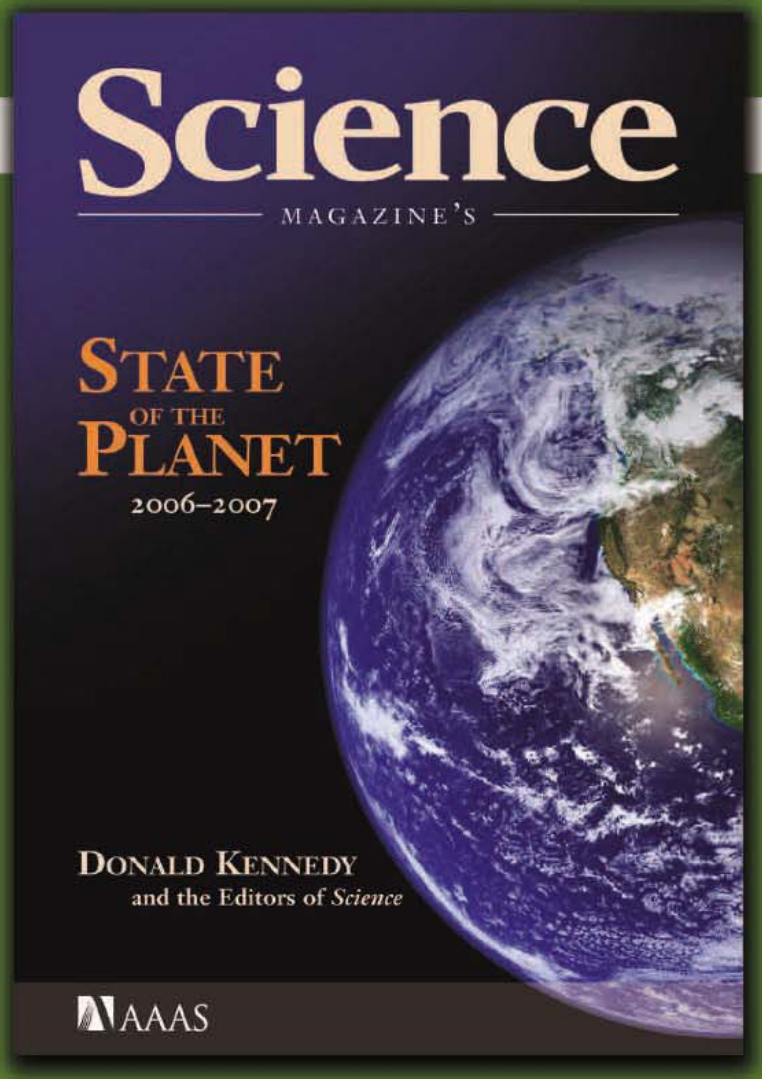


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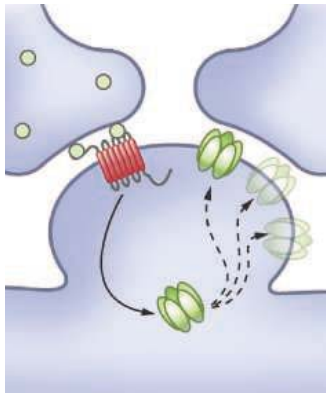

Increasing synaptic glutamate receptors.

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**PERSPECTIVE: Where Do You Think You Are Going? The NMDA-D1 Receptor Trap**
*C. Cepeda and M. S. Levine*
Activated NMDA receptors can trap D1 dopamine receptors in dendritic spines.

**TEACHING RESOURCE: Assembly and Organization of Macromolecular Complexes**
*M. Diversé-Pierluissi*
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**NEWS FOCUS: In Praise of Insulin Resistance**
*M. Leslie*
Immune-cell metabolic defect might hinder atherosclerosis.

**CLASSIC PAPER: Effects of Food Restriction on Aging—Separation of Food Intake and Adiposity**
*D. E. Harrison, J. Archer, C. M. Astle*
Genetically obese mice display extended longevity on a food-restricted diet; *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1835 (1984).

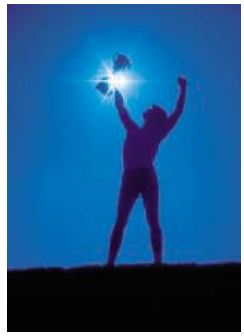
## SCIENCENOW

www.sciencenow.org DAILY NEWS COVERAGE

**High-Mileage Black Holes**
Supermassive black holes are found to be so energy efficient, they put hybrids to shame.

**Sticky Brains Don't Dull Memories**
Mutation in mouse gene prevents Alzheimer's symptoms, despite brain plaques.

**You Scratch My Back, I'll Scratch His**
Lopsided relationships can be beneficial to ecosystems.



Ups and downs of grad school success.

## SCIENCE CAREERS

www.sciencecareers.org CAREER RESOURCES FOR SCIENTISTS

**MISCINET: Educated Woman, Chapter 50—Superstar or Falling Star?**
*M. P. DeWhyse*
Scientific success can bring graduate students a sense of well-being, but it can also have a dark side.

**GLOBAL: Living and Working in France—Feature Index**
*E. Pain*
Part 2 of our feature shares personal experiences of European and American researchers in France.

**EUROPE: Experiencing France**
*A. Forde*
France is an attractive professional destination for scientists, as three European researchers can attest.

**US: Vin, Pain, and Science**
*J. Austin*
American scientists go to France for all sorts of reasons, but the most important reasons are scientific.

**US: Neural Prosthetics**
*V. Chase*
Christa Wheeler found the perfect field to meld her interests in medicine and body mechanics.

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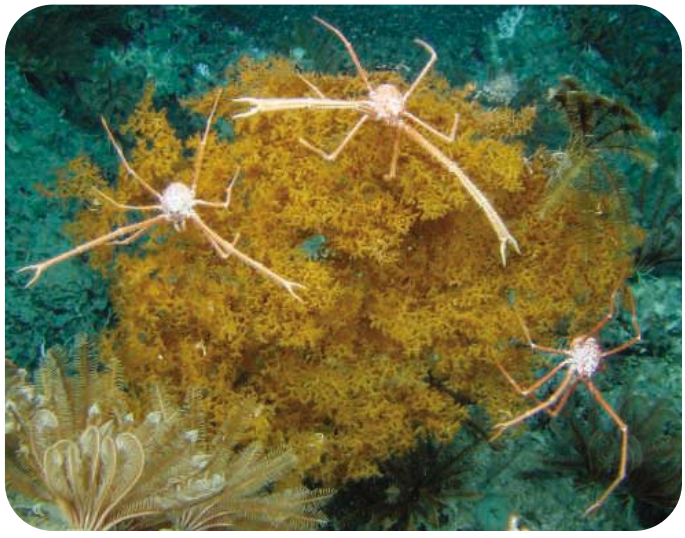
EDITED BY STELLA HURTLEY AND PHIL SZUROMI

## Corals in Deep Water

Tropical, shallow-water coral reefs have been the subject of intense research for many decades. The deepwater coral ecosystems, many of which occur at higher latitudes, are much less well known. **Roberts *et al.*** (p. 543) review the latest research on coldwater corals, focusing particularly on the North Atlantic, where most of the recent exploration has taken place. Like their shallow-water counterparts, deepwater coral reefs appear to harbor a high diversity of species. Much remains to be discovered about the biology of these systems, but it is already clear that they are vulnerable to threats from exploitation and climate change.



## Periodic Pulsing

Pulsars are spinning neutron stars with strong magnetic fields that generate radio beams that sweep across the sky. Why do some neutron stars emit radio waves but others do not? **Kramer *et al.*** (p. 549, published online 2 February; see the Perspective by **van den Heuvel**) found a pulsar, B1931+24, that looked normal for about 1 week but then suddenly switched off. It remained undetectable for 1 month before switching on again. These on-off cycles repeat. All pulsars spin more slowly as they lose energy, but B1931+24 spins down 50% faster when it is switched on. This behavior implicates particle currents and winds in pulsar deceleration, and allows the sizes of the currents to be measured.

## Teaching Spins to Stay

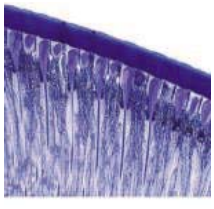
Manipulation of the spin state of quantum dots could provide a route for quantum information processing. However, it has been difficult to prepare the quantum dot in a particular state (either spin-up or spin-down), and then maintain that spin state because of internal scattering and spin-flip processes occurring within the dot. **Atatüre *et al.*** (p. 551) laser-cooled an electron spin on a quantum dot from 4 kelvin to 20 millikelvin and showed that its desired spin state can be achieved with 99.8% fidelity.

## A Super Seismically Slow Silicate

The ultralow seismic velocities seen for the core-mantle boundary are normally attributed to the presence of melted mantle. The main solid phase recently identified as stable, under the temperature and pressure conditions of this region, is a magnesium-rich silicate called post-perovskite. **Mao *et al.*** (p. 564) show through high-pressure experiments that seismic velocities in iron-rich post-pervoskite, which might be produced in mantle regions near the iron core, are slower even than those of ultraslow velocity waves. Thus, a mixture of solid phases that includes iron-rich post-perovskite might explain the seismic observations without requiring the presence of a melt.

## Imitating Insect Eyes

The eye of a bee contains thousands of integrated optical units that are pointed in different directions. Each of these units collects incident light from a narrow angular range and helps contribute to the eye's wide field of view. Through a combination of micro- and nanofabrication techniques, **Jeong *et al.*** (p. 557) made a synthetic analog that closely parallels these compound eyes and shows comparable optical properties.



## Closer Comet Cache

Comets are believed to be primitive dirty snowballs that come from the cold outer reaches of the solar system. However, **Hsieh and Jewitt** (p. 561, published online 23 March; see the Perspective by **Fitzsimmons**) propose that a new class of comets exists in the main asteroid belt. A survey of main-belt asteroids revealed three with cometary tails, which suggests that icy asteroids can become activated and appear as comets after collisions. As these objects likely formed in situ in a warmer environment, such main belt comets should differ in composition as well as orbit from the cold Kuiper Belt and Oort Cloud comets. Main belt comets could have contributed water to the early Earth.

## Cultural Recalibration

Comparison of major events in early Mediterranean cultures in Crete, the Levant, Egypt, and elsewhere during the Bronze Age requires an accurate chronology for comparison. One critical tie point is the age of the Santorini eruption, which flung ash across the area, but this needs to be augmented with longer and better chronologies in each locality. **Manning *et al.*** (p. 565) present a large number of radiocarbon dates spanning 300 years that, along with a more firm Santorini age (see the Brevia by **Friedrich *et al.*** and the cover), shift the Aegean record about 100 years earlier. Thus, the major New Palace Crete culture was contemporaneous with one in the Levant, not with the New Kingdom period of Egypt as had been inferred.

## Unreliable Mitochondrial DNA

Variability in mitochondrial (mt)DNA is often used to infer population size, history, and diversity on the assumption that mtDNA is essentially evolutionary neutral. **Bazin *et al.*** (p. 570; see the Perspective by **Eyre-Walker**) compared a wide range of animal species for polymorphisms in allozymes, nuclear DNA, and mtDNA. Within-species allozyme and nuclear DNA variability correlated with expected species abundance and ecological variables, whereas essentially no difference was observed between a broad range of taxa in terms of mtDNA variability. Instead, mtDNA seem to have undergone recurrent fixation of beneficial

CREDITS (TOP TO BOTTOM): AWI & IFREMER (2003); JEONG *ET AL.*

mutations and loss of variability at linked loci. Thus, mtDNA is far from a neutral marker; its diversity is essentially unpredictable and may not reflect population history and demography.

## Mosquito Resistance

What happens to malaria parasites in their wild mosquito vector? **Riehle *et al.*** (p. 577) examined wild mosquitoes fed on the blood of naturally infected people in Mali and identified four genes that affect the insects' ability to resist the parasite. The genes act against at least three different species of malaria parasite. One of the genes, which causes parasite melanization in the lab, probably has little effect in natural systems. The three other genes, however closely resemble pattern-recognition resistance genes found in a many plants and animals. A large proportion of wild mosquitoes remained uninfected despite being fed malaria-infected blood.

## Fungi Versus Plants and Mammals

Rice blast is an economically important disease caused by the fungus *Magnaporthe grisea*, which enters leaves by developing specialized structures called appressoria. **Veneault-Fourrey *et al.*** (p. 580) show that during invasion, the fungus undergoes a form of programmed cell death that involves autophagy. Thus, fungal pathogens can use cell death for cellular differentiation and remodeling during host infection. Fungal virulence, the ability of opportunistic fungal pathogens to thrive in mammals, is associated with a transformation from a filamentous, pseudohyphal form that grows at 25°C into a yeast form at 37°C. Using the plant pathogen *Agrobacterium tumefaciens* as a tool for T-DNA insertional mutagenesis, **Nemecek *et al.*** (p. 583) identified mutants that locked the organism in the filamentous form. One mutant that could not make the yeast form also showed defects in cell-wall formation, sporulation, and expression of virulence factors. The defect lay in a gene encoding a histidine kinase, which appeared to be the global regulator for morphological switching and virulence in several species of dimorphic fungi.

## Voltage-Gated Proton Channel

Voltage sensor domains comprise four transmembrane segments (S1 to S4) and are responsible for sensing changes in membrane potential and controlling gating of the pore domain (S5 and S6) in voltage-gated ion channels. **Sasaki *et al.*** (p. 589, published online 23 March) have identified a protein consisting primarily of a voltage-sensor domain (VSD) that appears to mediate voltage-gated proton currents. The proton currents exhibit pH-dependent gating and are sensitive to zinc ion concentrations, features that are characteristic of voltage-gated proton channels.

## BoTox Receptor

Botulinum neurotoxin type A (BoNT/A) is one of seven neurotoxins produced by the bacterium *Clostridium botulinum*. BoNT/A has a long half-life within cells and is widely used in treatments of wrinkles to chronic pain. Moreover, BoNT/A can cause paralysis that persists for months. BoNT/A is known to block neurotransmission by cleaving the protein SNAP-25 in presynaptic terminals, but it is not clear how this toxin selectively recognizes and enters neurons. **Dong *et al.*** (p. 592, published online 16 March; see the Perspective by **Miller**) now identify a protein component of the cellular receptor for BoNT/A as a synaptic vesicle protein, SV2. BoNT/A enters neurons via recycling synaptic vesicles by binding to SV2 isoforms, and cells and animals lacking SV2 are resistant to intoxication.

## Switching Spermatogenesis Off and Oogenesis On

Male and female germ cells enter meiosis at different times. Spermatogenesis results from meiosis during fetal development, whereas oogenesis results when meiosis initiates after birth. It has been thought that germ cells enter meiosis and initiate oogenesis by default, unless blocked by an uncharacterized diffusible signaling molecule produced by the testis. **Bowles *et al.*** (p. 596, published online 30 March) now show that retinoid metabolism inhibits meiosis in male embryos. In both males and females, the morphogen retinoic acid is produced in the mesonephric tubules for the initiation of meiosis. The morphogen is not degraded in the ovary, but it is specifically degraded in the testis by the p450 cytochrome enzyme CYP26B1.

CREDIT: NEMECEK *ET AL.*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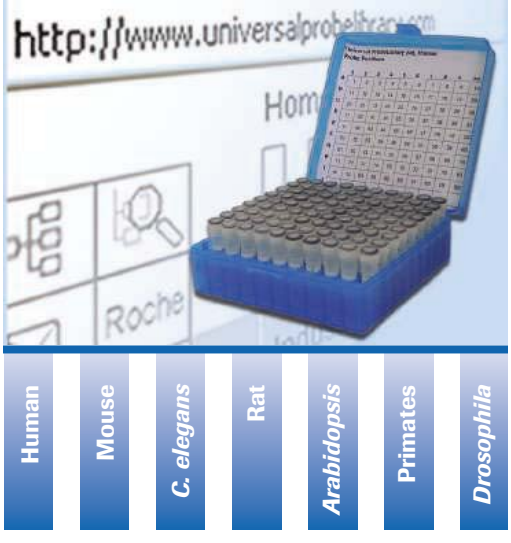


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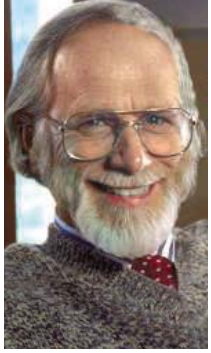


J. Michael Bishop, chancellor and professor at the University of California, San Francisco, is a member of the Joint Steering Committee for Public Policy (JSCPP; www.jscpp.org) and the NIH Director's Advisory Council.

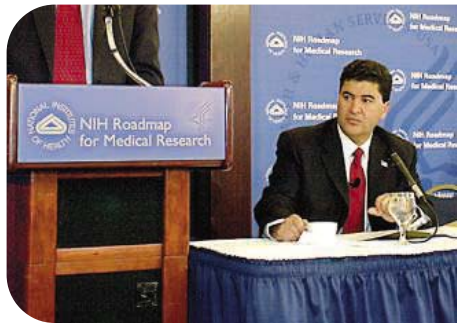
Harold Varmus, president of the Memorial Sloan-Kettering Cancer Center, is chair of the JSCPP and a former director of NIH.

# Re-Aim Blame for NIH's Hard Times

ANXIETY AND ANGER ARE RIFE AMONG THE BIOMEDICAL RESEARCH COMMUNITY OVER THE dwindling fortunes of the National Institutes of Health (NIH). The anxiety is justified: Success rates for grant applications have fallen, on average, from over 30% in 2003 to under 20% (and to even less at some Institutes), and the Bush administration's budget projections imply further declines. But the anger is another matter: Much of it is mistakenly directed at NIH itself and threatens to undermine the credibility of the agency with both its federal patrons and its public constituencies.

Between 1999 and 2003, NIH enjoyed extraordinary largesse as Congress and two successive administrations doubled its budget to about $27 billion. During this period, as expected, NIH awarded more multiyear grants, committing itself to increasing fiscal obligations in the ensuing years. At the same time, the average grant size grew beyond the rate of inflation and the number of applications also rose significantly.

After such expansion, a gradual decline toward more customary increases is required to ensure that substantial uncommitted funds are available for new grants. But the hoped-for "soft landing" did not occur. Most federal budgets, including NIH's, have flattened in the service of larger budgetary agendas, such as tax cuts and financing the war in Iraq. Congress has turned a skeptical eye on NIH, demanding to know at an unrealistically early stage what exceptional benefits the doubling has brought to those suffering from diseases and asking why NIH cannot prosper with its doubled budget. Now, facing its third consecutive year of sub-inflationary increases, NIH is likely to have 11% less spending power in 2007 than it did in 2004.




Rather than galvanizing political action to restore at least inflationary budgetary increases, these developments have precipitated an irrational response from some members of our research community. They have begun to blame the agency itself, accusing the NIH administration of mismanagement and ill-conceived adventures.

The favorite whipping boy is the recently developed NIH Roadmap. The contents of the Roadmap were shaped a few years ago by extensive consultations with extramural scientists, not invented unilaterally by the NIH leadership, and represent a response to converging forces, including demands from Congress—and from diverse physicians, disease-research advocates, and scientists—for a greater sense of mission, more risk-taking, and expanded interdisciplinary research. In its first couple of years, the Roadmap has launched laudable programs, supported mainly by highly competitive awards to individual investigators, to encourage creative but high-risk research (the Pioneer Awards); new approaches to biomedical computing, structural biology, nanomedicine, and chemical biology; and a reconfiguring of the infrastructure for clinical research.

Despite its high ambitions, the Roadmap has required no more than a modest 1.2% of the NIH budget. "Shelving" the Roadmap, as called for by one recent commentary,* would not heal NIH's financial maladies. But it just might persuade Congress and other potential critics that members of the biomedical research community are hopelessly inured to change and less concerned about the commonweal than the professional well-being of scientists.

What then is to be done? First, stop blaming NIH—it is a victim, not a culprit, and it urgently needs our collective help. Second, redirect the hue and cry to Congress and the White House. Professional societies and disease-advocate groups have taken up the cause, but investigators in the trenches have been singularly silent. And third, support NIH in its efforts to manage resources prudently: Understand the nature of its difficulty and the rationale for restricting the size of awarded grants; encourage favored treatment of applications from scientists seeking their first awards; and accept opportunities to provide advice by serving on NIH's advisory and review panels.

This is a time for concern and action, not despair. Biomedical research has found itself in seemingly dire straits before, yet recouped rapidly when Congress learned that the health sciences were adversely affected by budgetary shortfalls.† NIH still has potent allies in Congress. The public enthusiastically supports health research and recognizes that modern science is making rapid progress against feared diseases. Scientists should reinforce those alliances by making common cause with the leadership of NIH, rather than unjustly undermining its credibility.

**– J. Michael Bishop and Harold Varmus**

**J. Clin. Invest.* **116**, 844 (2006). †*N. Engl. J. Med.* **354**, 1665 (2006).

10.1126/science.1128904

CREDIT (RIGHT): RICK KOZAK

**The Sahel landscape.**

EARTH SCIENCE

## Drying Out

The semiarid Sahel region, which bridges the Sahara desert and the savanna landscape in Africa, has endured multiple extreme droughts since the 1960s. Loss of vegetation has been attributed in part to periods of reduced rainfall, but the long-term contribution of livestock grazing to local desertification is still debated. Recent studies have interpreted satellite data to support a greening process, or recovery of vegetation, since rainfall began to increase in the mid-1980s, suggesting that grazing has had minimal lasting impact on the landscape.

Hein and De Ridder argue that the satellite images have been systematically misinterpreted because of a flawed core assumption that rainfall variation would not alter rain-use efficiency (RUE): the ratio of annually generated plant material to rainfall. By analyzing data from six semiarid sites, they find that RUE instead appears to vary quadratically with rainfall. Correcting for this phenomenon suggests that anthropogenic degradation of the Sahel vegetation cover is a likely factor in the magnitude of the droughts over the past 40 years and suggests that future droughts may have a stronger impact than previously projected. — HJS

*Global Change Biol.* **12**, 10.1111/j.1365-2486.2006.01135x (2006).

MATERIALS SCIENCE

## Fine Lines in Glass

The feature resolution attainable using photolithography has generally been limited by the wavelength of the incident light. However, as light sources approach the extreme ultraviolet (EUV), the polymer resists become the limiting factor because etching leaves behind rough edges, probably due to polydispersity. A promising alternative is to fabricate resists from amorphous films composed of small organic molecules with high glass-transition temperatures. In this vein, Chang *et al.* prepared films with glass transitions at ~120°C from derivatives of *C*-4-hydroxyphenyl-calix[4]resorcinarenes. A fluorinated photoacid was incorporated to solubilize local calixarenes on exposure to light, resulting in a positive-tone resist. The authors optimized the material by varying the extent of calixarene hydroxyl protection with bulky *tert*-butyloxycarbonyl (*t*-Boc) groups. At 70% *t*-Boc incorporation, EUV irradiation produced lines with 30 nm resolution. Moreover, a line-edge roughness below 5 nm was obtained for 50-nm lines. — PDS

*J. Mater. Chem.* **16**, 1470 (2006).

NEUROSCIENCE

## Replenishing the Sheath

After spinal cord injury, neuronal axons may survive; however, they often lose their myelin sheath, which is necessary for impulse conduction, and remyelination does not occur. Because of the ability of adult neural precursor cells (NPCs) to self-renew and to differentiate into multiple cell types, they serve as a potential source of cells to repair central nervous system injuries.

Karimi-Abdolrezaee *et al.* have examined the ability of mouse NPCs to integrate with injured spinal cord tissue in rats that have been injured at the mid-thoracic level by aneurysm clip compression of the spinal cord. Adult NPCs from the mouse brain were transplanted, and growth factors, an anti-inflammatory drug, and an immunosuppressant were infused into the spinal cord of rats at 2 weeks after trauma, representing the subacute phase of spinal cord injury. This transplantation method promoted the survival and/or differentiation of adult neural progenitors with an oligodendrocyte lineage and resulted in remyelination of injured axons. Locomotion function and hindlimb movement improved after treatment with NPCs in the subacute model. These findings may lead to insights into spinal cord injury and therapeutic intervention. — BAP

*J. Neurosci.* **26**, 3377 (2006).

APPLIED PHYSICS

## Mass-Producing SET Sensors

Weak electric fields at surfaces, whether in a solid-state device or a frozen cell section, can be mapped out noninvasively by mounting a single-electron transistor (SET) onto a scanning probe platform. However, the designs recently used to implement these scanning SETs have several drawbacks. Because the devices are easily damaged, elaborate methods for producing them one at a time are inefficient; moreover, the need for extremely low-temperature (<1 K) operating conditions, as well as laser-based feedback, limits the range of samples amenable to study.

Brenning *et al.* have fabricated SETs on the ends of silicon nitride cantilevers, which in turn are mounted on rigid quartz crystal resonators. These noncontact atomic force microscopy tips use the change in resonant frequency as the feedback signal and scan at heights of a few nanometers. More than 200 tip assemblies can be fabricated at a time via electron-beam lithography, and they have large enough charging energies to operate at pumped liquid helium temperatures. The authors demonstrate the device by scanning a $SiO_2$ surface at 4.2 K. — PDS


**Cantilever-mounted single-electron transistor (D, drain; G, gate; S, source).**

*Nano Lett.* **6**, 10.1021/nl052526t (2006).

PSYCHOLOGY

## A Bad Outcome Implies Intent

The last storyline on a once-popular television show described the prosecution of four defendants under the Good Samaritan law on the grounds that they had failed to act to prevent

CREDITS (TOP TO BOTTOM): FRANK KROENKE/PETER ARNOLD, INC.; BRENNING *ET AL.*, *NANO LETT.* **6**, 10.1021/NL052526T (2006)

harm. The capacity to form judgments of morality (good/bad or helpful/harmful) and of intentionality (an outcome brought about deliberately/accidentally) has been one of the experimentally accessible aspects of investigations into how and when children develop a theory of mind and an understanding of causality.

Leslie *et al.* have combined these two themes in a study of when children exhibit an adult-like asymmetry in making a distinction between a harmful side effect, which grown-ups commonly think of as being intentional and hence morally suspect, and a good side effect, which is usually regarded as an unintentional consequence of the action. They find evidence for this behavior, which they call the side-effect effect, in 4- and 5-year-olds but not in 3-year old children. In the specific scenario tested, that of Janine who disliked/liked a frog brought over by Andy, who did not care about her feelings about frogs, the older children were abler in correctly grasping his indifference, and then attributing purposefulness to the bad outcome but not the good one. — GJC

**Girl and the frog.**

*Psychol. Sci.* **17**, 422 (2006).

CHEMISTRY

## Sorting Sulfides

The abundant organosulfur compounds in crude oil are oxidized to acidic pollutants (such as sulfuric acid) during combustion. To minimize their environmental impact, gasoline and diesel are subjected to desulfurization processes before use. However, tighter regulations have spurred chemists to pursue more efficient desulfurization methods, which would treat heavy oil before the cracking process that yields transportation fuels.

Toward this end, Choudhary *et al.* present a screening method to differentiate and quantify the organosulfur components of heavy oil. They first assay the aliphatic compounds by selective oxidation, followed by chromatographic/mass spectral analysis of the aromatics. Components are classified based on size and structure (mono- to hexacyclic, compact or extended geometry), and the relative reactivities of each class are then compared under varying desulfurization conditions. They find, for example, that phenanthrothiophenes are the least reactive toward hydrogenolysis (reductive removal of the sulfur as $H_2S$) at 622 K but relatively more reactive at 655 K. They also determine which aromatics accept hydrogen more rapidly at carbon than at sulfur. These data offer useful projections for large-scale process optimizations. — JSY

*Angew. Chem. Int. Ed.* **45**, 10.1002/anie.200503660 (2006).

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## << A Proton Gradient Signals Asymmetry

Adams *et al*. identified the $H^+$-V-ATPase, which is a vacuolar and plasma membrane proton pump, in a pharmacological screen of *Xenopus* embryos in which defects in left-right asymmetry (heterotaxia) were scored. Inhibition of the $H^+$-V-ATPase with drugs such as concanamycin or expression of a dominant-negative $H^+$-V-ATPase subunit resulted in heterotaxia and the loss of asymmetric expression of one of the first genes with asymmetric expression, *Nodal*, suggesting that $H^+$-V-ATPase provides a very early asymmetry signal indeed. Proton pump subunits were more abundant on the right side of the embryo as early as the two-cell stage, and proton efflux was greater on the right side of the embryo. In addition, the right side of the embryo was hyperpolarized relative to the left side, as expected from the electrogenic nature of the $H^+$-V-ATPase. Elimination of asymmetric $H^+$ flux by expression of a symmetrically localized plasma membrane $H^+$ pump or exposure of the embryos to low pH, or elimination of the hyperpolarization of the membrane by incubating the embryos with palytoxin, both produced heterotaxia. This suggests that the activity of the $H^+$-V-ATPase produces asymmetry through a combination of an effect on pH and the membrane potential. A role for $H^+$-V-ATPase in asymmetry was also noted for chick and zebrafish embryos and appeared to serve as one of the earliest signals for asymmetry. Disruption of $H^+$-V-ATPase activity randomized the expression of *Nodal* and *Shh* in chicks, and in zebrafish $H^+$-V-ATPase activity was required for asymmetric expression of *Southpaw* and before formation of the Kupffer's vesicle, a ciliated organ involved in organ asymmetry. — NRG

*Development* **133**, 1657 (2006).

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| science_letters@aaas.org | (for queries about letters) |
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***Science* International**

**Europe** (science@science-int.co.uk) **editorial: international managing editor** Andrew M. Sugden; **senior editor/perspectives** Julia Fahrenkamp-Uppenbrink; **senior editors** Caroline Ash (Geneva: +41 (0) 222 346 3106), Stella M. Hurtley, Ian S. Osborne, Stephen J. Simpson, Peter Stern; **associate editor** Joanne Baker **editorial support** Alice Whaley; Deborah Dennison **administrative support** Janet Clements, Phil Marlow, Jill White; **news: international news editor** Eliot Marshall **deputy news editor** Daniel Clery; **correspondent** Gretchen Vogel (Berlin: +49 (0) 30 2809 3902, FAX +49 (0) 30 2809 8365); **contributing correspondents** Michael Balter (Paris), Martin Enserink (Amsterdam and Paris), John Bohannon (Berlin); **intern** Laura Blackburn

**Asia** Japan Office: Asca Corporation, Eiko Ishioka, Fusako Tamura, 1-8-13, Hirano-cho, Chuo-ku, Osaka-shi, Osaka, 541-0046 Japan; +81 (0) 6 6202 6272, FAX +81 (0) 6 6202 6271; asca@os.gulf.or.jp; **asia news editor** Richard Stone +66 2 662 5818 (rstone@aaas.org) **japan news bureau** Dennis Normile (contributing correspondent, +81 (0) 3 3391 0630, FAX 81 (0) 3 5936 3531; dnormile@gol.com); **china representative** Hao Xin, + 86 (0) 10 6307 4439 or 6307 3676, FAX +86 (0) 10 6307 4358; haoxin@earthlink.net; **south asia** Pallava Bagla (contributing correspondent +91 (0) 11 2271 2896; pbagla@vsnl.com)

EXECUTIVE PUBLISHER **Alan I. Leshner**
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**Fulfillment & Membership Services** (membership@aaas.org) **director** Marlene Zendell; **manager** Waylon Butler; **systems specialist** Andrew Vargo; **specialists** Pat Butler, Laurie Baker, Tamara Alfson, Karena Smith, Vicki Linton; **circulation associate** Christopher Refice

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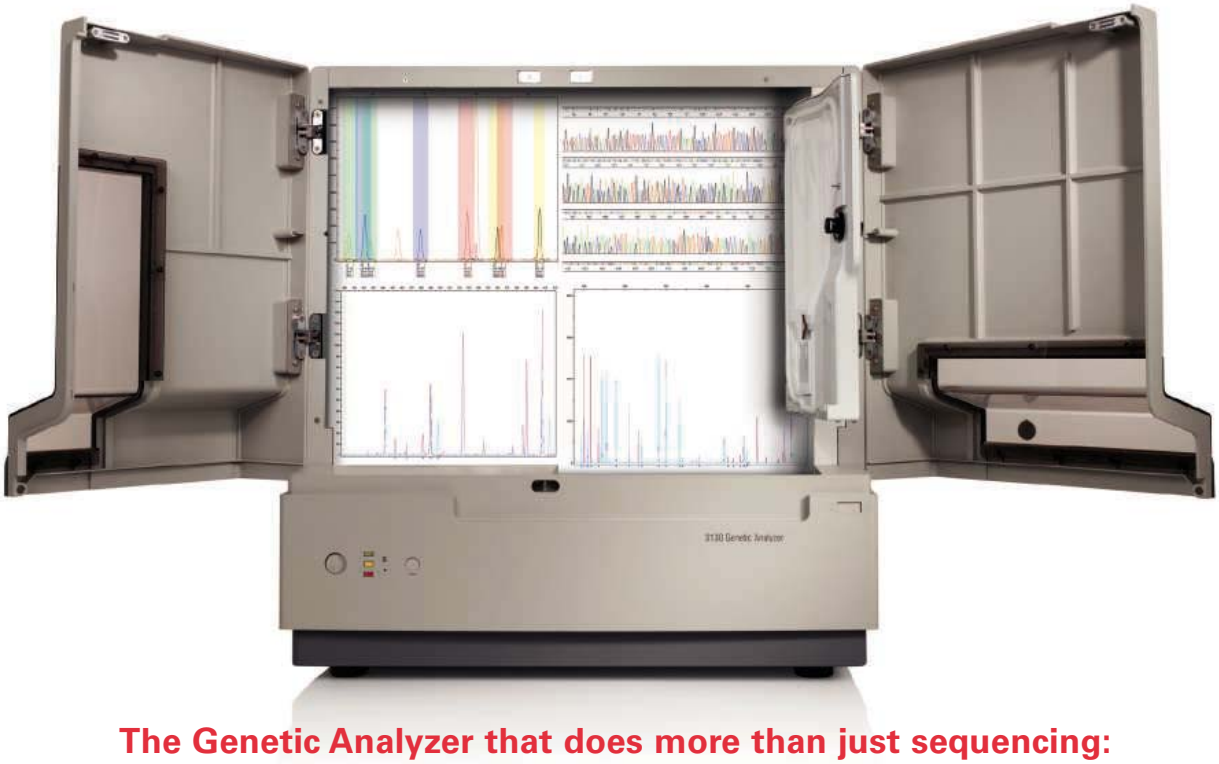

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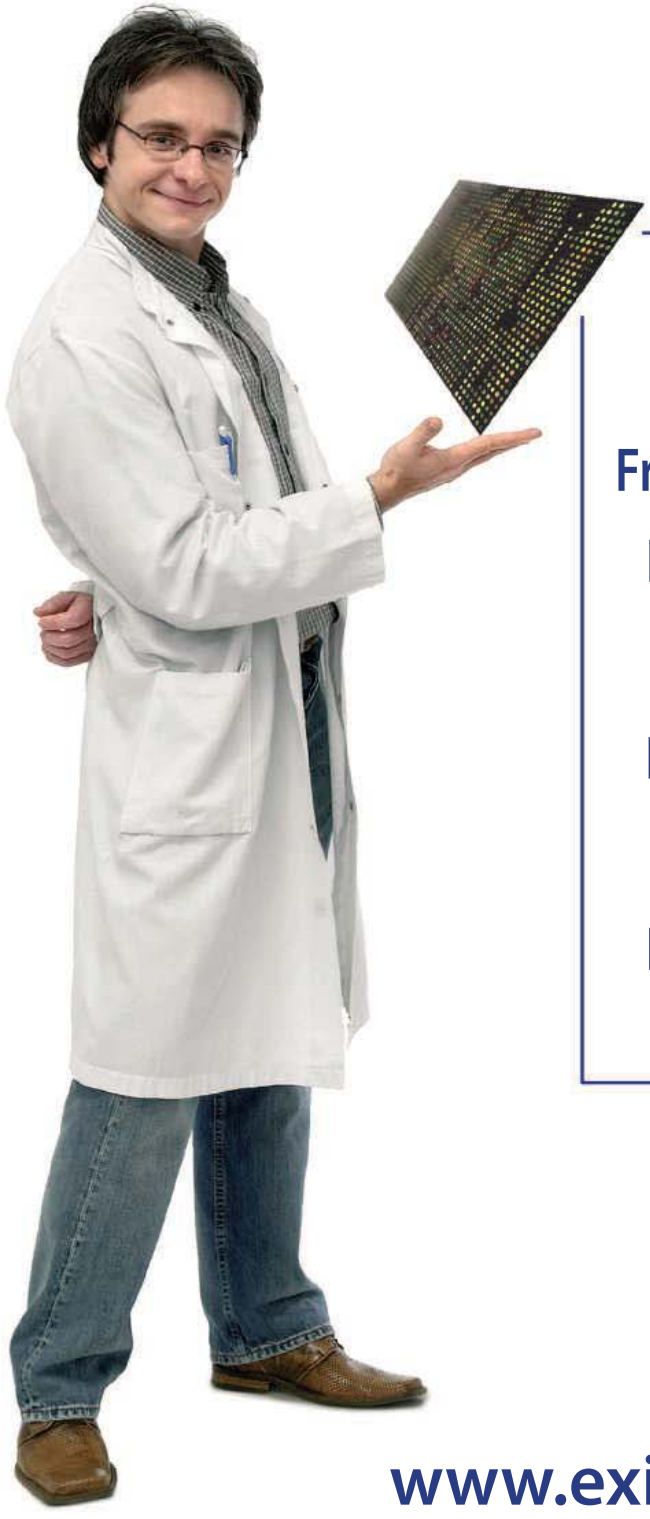

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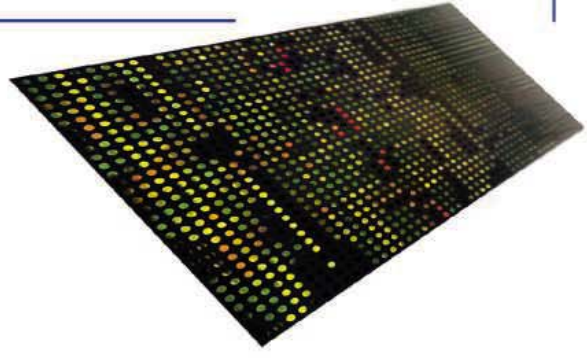

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EDUCATION

## Mashing Moon Myths

To conspiracy theorists, this photo of Apollo 16 Commander John Young in midjump furnishes telling evidence that NASA faked the moon landings in the 1960s and 1970s. Why does the flag seem to be flapping when the moon has no atmosphere, they demand, and where is Young's shadow if the only illumination is sunlight from the viewer's left? At Moon Base Clavius, systems engineer Jay Windley of Salt Lake City, Utah, dissects the lunar hoax arguments, which are still circulating. A strength is Windley's meticulous analysis of photos and video. The wrinkles and creases in the flag cause its apparent motion, he notes. And the edge of Young's shadow—which is offset because he's above the surface—is visible at the right of the photograph. >> www.clavius.org

EDUCATION

## What Tortured the Artist?

Vincent van Gogh (1853–1890) endured frequent mental breakdowns and killed himself not long after painting the hallucinatory *Starry Night* (below). Hypotheses for his instability include bipolar disorder and poisoning from drinking absinthe. At The Illness of Vincent van Gogh, biochemist Wilfred Niels Arnold of the University of Kansas Medical Center in Kansas City lays out the case for an alternative diagnosis: acute intermittent porphyria. In this inherited metabolic disorder, noxious compounds accumulate because the body's production line for heme—a key component of hemoglobin—falters. With its embedded questions and lecture format, the site is geared toward medical students, but any curious visitor can gain insight into the painter's condition. >> www.med.wayne.edu/elab/vangogh/MainIndex.htm

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WEB LOGS

## All Physics, All the Time

Don't have time to check all of your favorite physics blogs? Neither did undergraduate Jeff Hodges of Bowling Green State University in Kentucky, so he created the compilation Mixed States. Every hour, the site automatically gathers the latest posts from more than 80 Web logs and physics news collections. You can snag headlines from PhysicsWeb, ponder quantum chromodynamics with the folks at Life on the Lattice, and probe the confluence of physics and biology with the BioCurious group, all without straying from the site. >> mixedstates.somethingsimilar.com

COMMUNITY SITE

## Do I Know You? >>

You can usually recognize a friend even if he changes his facial expression, dons a hat and dark glasses, or grows a beard. Teaching machines to be equally discerning might help thwart terrorists and criminals and clarify how our brains perform the feat. The Face Recognition Homepage from computer scientist Mislav Grgic of the University of Zagreb in Croatia and colleague Kresimir Delac is a hub for researchers in the field. You'll find links to more than 20 databases that hold facial photos for testing machine perception. The site also gathers PDFs of papers that describe face-recognition algorithms and highlights new and classic articles. Other resources include a roster of companies working on identification systems and a calendar of upcoming conferences. >> www.face-rec.org

RESOURCES

## Plants Under Pressure

Heat, drought, salt buildup, cold, and other forms of adversity shrivel agricultural production in many parts of the world. One clearinghouse of information on these environmental conditions and how crops respond to them is Plant Stress, curated by emeritus researcher Abraham Blum of the Volcani Center in Israel. Backgrounders explain the effects of nine plant stresses and explore methods for alleviating their impact. For instance, solutions for saline soil include hauling away the contaminated dirt and genetically engineering crops for salt resistance. Plant Stress has also sprouted a news section that notes fresh research findings, a bibliography, and how-tos on more than a dozen techniques for studying suffering plants. >> www.plantstress.com

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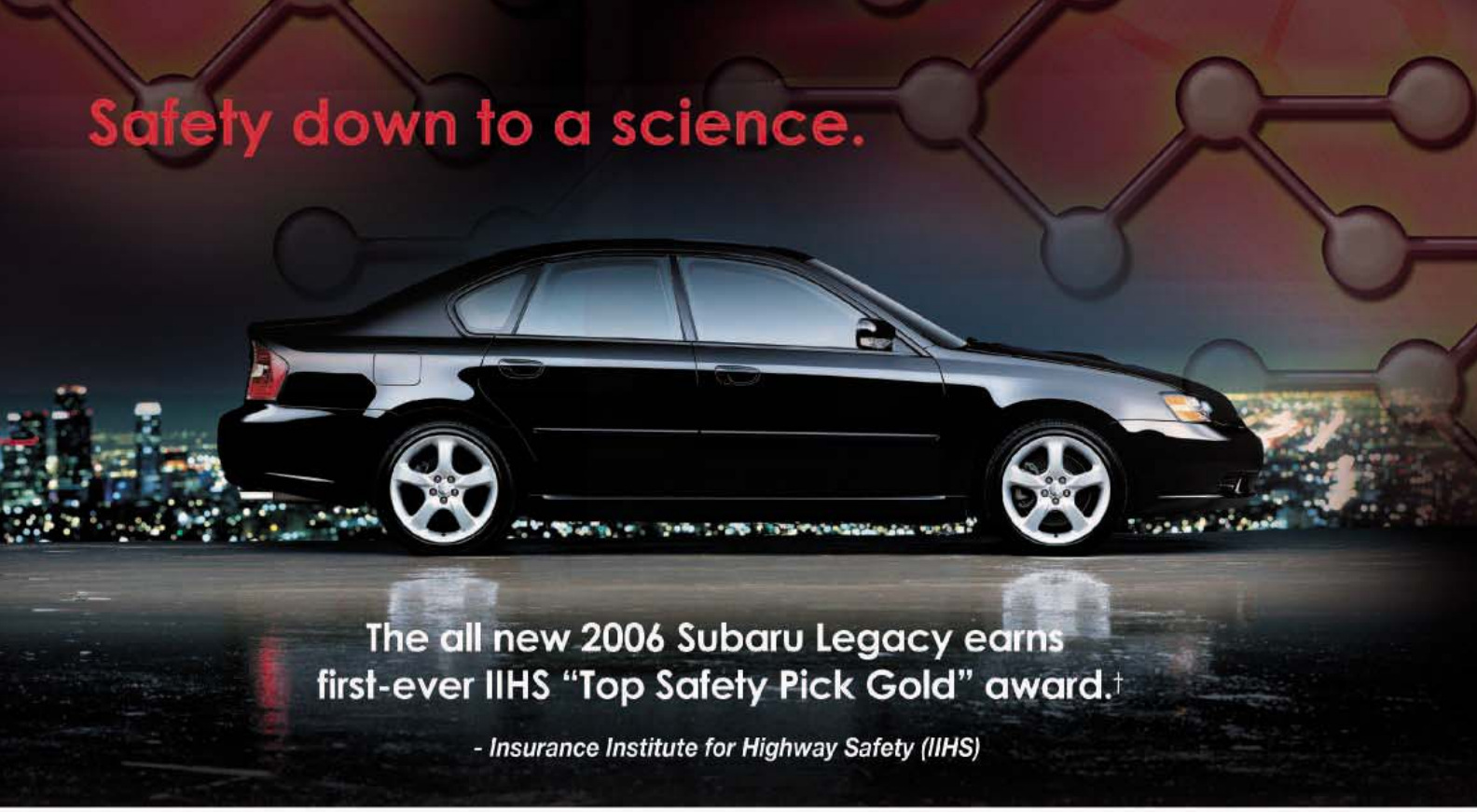


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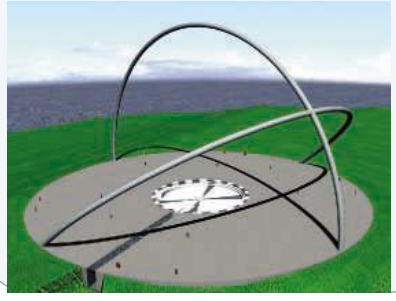
# From Coal to the Stars

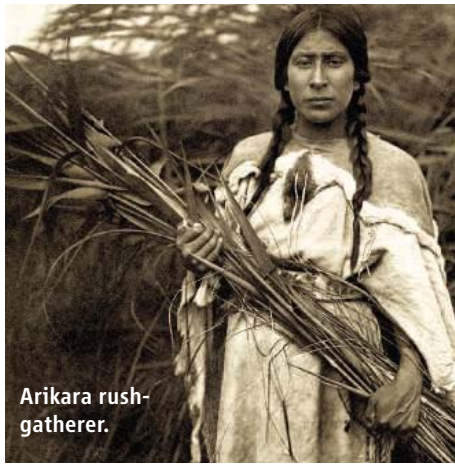
A section of the Ruhr district in northwestern Germany, once one of the most heavily industrialized areas in Europe, is being turned into an astronomy park. Below is Europe's first "horizon observatory," slated to rise on a rehabilitated slag heap, part of a former coal mine.

The artificial hill in the otherwise flat landscape provides a rare unobstructed view of the horizon, says Daniel Brown of Liverpool John Moores University in the United Kingdom, who presented the plan earlier this month at a meeting of the Royal Astronomical Society in Leicester, U.K. Fifty meter-high arches will help visitors orient to compass points, allowing them to observe how the sun and moon move with the seasons.

The observatory is part of a 140-hectare park being built around the slag heap by a group of astronomers, teachers, and private citizens, with support from the European Union. Already open is a giant sundial featuring an 8.5-meter obelisk. The park is scheduled for completion by the end of 2007.



# WORKING TO THE BONE >>



Arikara rush-gatherer.

In the Arikara tribe, which lived along the Missouri River in the Dakotas between the 14th and 19th centuries, the women did all the farming. Historical accounts relate that they produced so much corn by the 1850s that they had fat surpluses for trade.

Now Arikara bones have furnished direct testimony about their lives. Daniel Westcott of the University of Missouri, Columbia, and Deborah Cunningham of the Smithsonian Institution in Washington, D.C., examined between 95 and 160 pairs of male and female arm and leg bones from a period spanning nearly 4 centuries. They measured indications of mechanical load, including the area of the weight-bearing cortex and how bone cross sections departed from circularity.

The study, to appear in the July *Journal of Archaeological Science*, found that as agriculture intensified, women's leg bones changed. By the late 1700s, their left legs showed signs of having borne greater loads, which the authors suggest stemmed from "pushing off" on the left leg while working the fields. Anthropologist Christopher Ruff of Johns Hopkins University in Baltimore, Maryland, says this makes sense. "Lower limbs tend to be 'right dominant' in things like kicking a ball, but the left is used to stabilize the body, which is actually more stressful biomechanically."

While the women were in the fields, the men were developing a different asymmetry, the authors report: Their right arms became larger, probably as they relied increasingly on rifles rather than bows and arrows, which put stress on both arms.

# DEDICATED TO THE WORD

A man with epilepsy has supplied compelling evidence for an area in the brain dedicated to processing written words as entities, rather than letter by letter.

The region known as the visual word form area (VWFA) lights up when individuals read words, but its role has been controversial because it's also activated by faces or objects. Neuroscientists led by Laurent Cohen of the Hôpital de la Salpêtrière in Paris tested a man with severe epilepsy who was about to have a small area near the VWFA removed. Prior to the surgery, the man took 600 milliseconds to read common words. Scans and electrodes showed that the VWFA was activated when he read words, whereas different areas lit up when he named objects from pictures.

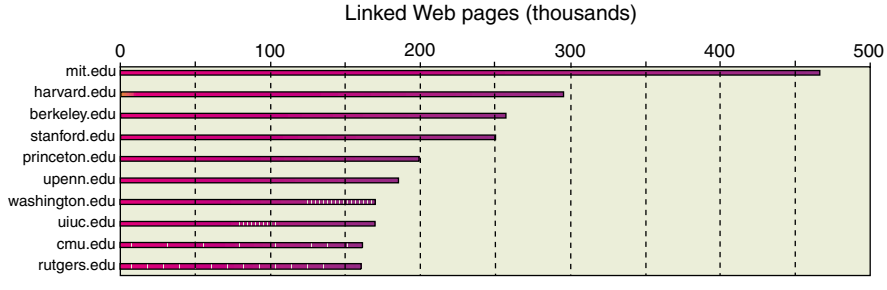
After the surgery, the patient could still identify objects quickly. But he took a full second to read a three-letter word. For every additional letter, his response time increased by about 300 milliseconds, suggesting he was reading letter by letter, the researchers report in the 20 April issue of *Neuron*.

Brain scans confirmed that the VWFA, disrupted by the surgery, no longer lit up at the sight of words. "It seems to be indispensable only for reading," Cohen says. Cognitive neuroscientist Alex Martin of the U.S. National Institute of Mental Health in Bethesda, Maryland, says the study offers "compelling and dramatic evidence" for a reading node in the brain. But he's mystified at the existence of such a specialized area for a task invented only 6000 years ago.

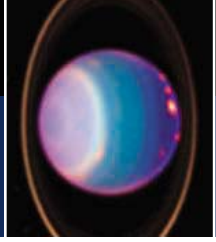
# The Superwired

The Massachusetts Institute of Technology is the academic world's biggest node by far in terms of Internet connectedness, according to a new ranking devised by Peter Hirst, a Boston-based science and technology consultant. Hirst took the first 300 from a ranking of 500 top universities produced annually by Shanghai Jiao Tong University in China and, using about a million Google searches, counted the number of Web pages linking to each university from the other 299. He came up with a new metric, the "G-factor."

Of the top 20 on the G-factor scale, the only non-U.S. institutions are Cambridge and Oxford universities and the Swiss Federal Institute of Technology. For more information, go to www.peterhirst.com




CREDITS (TOP TO BOTTOM): EDWARD S. CURTIS/COURTESY DARTMOUTH COLLEGE LIBRARY; THE ROYAL ASTRONOMICAL SOCIETY



Why Uranus tilts 512

The Jekyll-and-Hyde gene 515

ARCHAEOLOGY

# New Carbon Dates Support Revised History of Ancient Mediterranean

During the Late Bronze Age, the Aegean volcanic island of Thera erupted violently, spreading pumice and ash across the eastern Mediterranean and triggering frosts as far away as what is now California. The Theran town of Akrotiri was completely buried. Tsunamis up to 12 meters high crashed onto the shores of Crete, 110 kilometers to the south, and the cataclysm may ultimately have sped the demise of Crete's famed Minoan civilization. For nearly 30 years, archaeologists have fought over when the eruption took place. Those who rely on dates from pottery styles and Egyptian inscriptions put the event at roughly 1500 B.C.E., whereas radiocarbon experts have consistently dated it between 100 and 150 years earlier.

**Under the ash.** Akrotiri, buried by the eruption of Thera, is often called the "Pompeii of the Aegean."

Now, two new radiocarbon studies on pages 548 and 565 claim to provide strong support for the earlier dates. The studies "convincingly solve the problem of the dating of the Thera eruption," says archaeologist Colin Renfrew of Cambridge University in the United Kingdom, who was not involved in the work. If correct, the earlier dates would have "major consequences" for the relationships between Egypt, Minoan Crete, and Mycenaean Greece, says archaeologist Jeremy Rutter of Dartmouth College: "The issue of which direction artistic and other cultural influences was traveling may change significantly."

But many archaeologists who have long defended the later dates are unmoved. "I am not impressed," says Egyptologist Manfred Bietak of the University of Vienna in Austria, who prefers to rely on detailed Egyptian records for the same period. Archaeologists on both sides agree on one thing: The pottery found at Akrotiri since Greek archaeologists began excavating there during the 1960s has a distinctive style featuring spirals and floral motifs, known as Late Minoan IA (LM IA). The LM IA period also corresponds to what archaeologists consider the height of Minoan civilization. Because pottery was widely traded across the Mediterranean, sites that have pottery styles later than LM IA—such as Late Minoan IB, which features depictions of dolphins, octopi, and other sea creatures—must postdate the eruption. This makes it possible to construct relative chronologies for the region despite the debates over absolute dating.

One team, led by archaeologist Sturt Manning of Cornell University, dated 127 radiocarbon samples from Akrotiri and other Aegean sites thought—based on relative chronologies—to span a period from about 1700 to 1400 B.C.E. Manning and colleagues used a new radiocarbon calibration curve (described last year in the journal *Radiocarbon*) as well as sophisticated statistical models and cross-checked some samples among three different dating labs. They dated the eruption to between 1660 and 1613 B.C.E., within 95% confidence intervals.

That's a fairly close match to the findings of a second team, led by geologist Walter Friedrich of the University of Aarhus in Denmark. In 2002, Friedrich's graduate student Tom Pfeiffer found an olive branch, complete with remnants of leaves and twigs, that had been buried alive in pumice from the eruption. Radiocarbon dating fixed the death of the branch's outermost ring, and thus the eruption of Thera, between 1627 and 1600 B.C.E., again at 95% confidence levels. The authors of both papers argue that these earlier dates rule out the "conventional" chronology of about 1500 B.C.E.

"That is great news about the olive tree," says dendrochronologist Peter Kuniholm of Cornell, although he cautions that it is more difficult to assign specific years to the rings of a slender olive branch than to more commonly used trees such as conifers and oaks. Archaeologist Gerald Cadogan of the University of Reading, U.K., adds that the dates given by the two papers are "pretty consistent" and that their validity is bolstered because they are "put in context by other dates from before and after from elsewhere in the Aegean."

Manning and colleagues say the early dates suggest that the conventional linkage between Minoan and Egyptian chronologies, which puts the apex of Minoan civilization contemporaneous with Egypt's 16th century B.C.E. New Kingdom, is wrong. The New Kingdom, especially during the rule of Pharaoh Ahmose, was the high point of Egyptian power. Rather, the Minoans would have reached their own heights during the earlier Hyksos period, when the Nile delta was ruled by kings whose ancestors came from the Levant. Rutter says Egyptologists have tended to discount the importance of the Hyksos, whom Ahmose eventually chased out of Egypt: "The Hyksos have gotten lousy press."

CREDIT: S. W. MANNING *ET AL.*, *SCIENCE*

This chronological realignment would also mean that the famous gold-laden Mycenaean Shaft Graves—excavated by German entrepreneur Heinrich Schliemann in the late 1800s and known to correlate with the LM IA period as well as the beginnings of Mycenaean power in the Aegean—would also be contemporaneous with the Hyksos. Some archaeologists had speculated that the Mycenaeans owed their rise to a strategic alliance with the New Kingdom; the new radiocarbon dates would instead raise the possibility that they were allied with the Hyksos, Rutter says. At the very least, Manning says, "it would make the Hyksos world much more important and interesting." Manning adds that the earlier chronology would create "a different context for the genesis of Western civilization."

But many proponents of the later chronology are sticking to their guns. The radiocarbon dates create "an offshoot from the historical Egyptian chronology of 120 to 150 years," says Bietak. "Until the reasons for this offshoot are solved, we are chewing away at the same old cud."

**Buried treasure.** Excavations at Akrotiri have unearthed fabulous frescoes and distinctive pottery.

Bietak and others have argued that radiocarbon dating is not infallible and that the earlier date for the Thera eruption is contradicted by excavations in Egypt and on Thera itself. He and other archaeologists have found LM IA pottery in stratigraphic layers that Egyptian records date to later periods, and at Akrotiri they have unearthed a style of Cypriot pottery that apparently does not show up until the 16th century B.C.E. in Egypt.

"There are no current grounds for thinking that the Egyptian historical chronology could be out by more than a few years," says archaeologist Peter Warren of the University of Bristol, U.K. "This chronology has been constructed by hundreds of expert Egyptologists over many decades."

Nevertheless, Rutter says, the *Science* authors "have done what they can to overcome" the objections by advocates of a later date for Thera. And both sides agree that there is a lot at stake in the debate. Until it is resolved, Warren says, at least for the Late Bronze Age, "we would have to forget about serious study of the past and relationships between peoples." **–MICHAEL BALTER**

STEM CELLS

# Court Rules in Favor of California Stem Cell Institute

A California court has ruled that a $3 billion initiative for funding stem cell research does not violate the state's constitution. The ruling, a widely expected victory for California's research institutions, means that bond sales can proceed so that the California Institute for Regenerative Medicine (CIRM) can fund grants. But the plaintiffs plan to appeal, so CIRM may remain hamstrung for at least another year.

**Triumphant.** Robert Klein, chair of CIRM's board, is celebrating after a court ruled that California's stem cell initiative is constitutional.

CREDITS (TOP TO BOTTOM): ERICH LESSING/ART RESOURCES; KURT ROGERS/*SAN FRANCISCO CHRONICLE*/CORBIS

CIRM, created by Proposition 71 and approved by California voters in November 2004, was set up to fund research on human embryonic stem cells that is not eligible for federal support. The institute has gotten off to a slow start, however, because of lawsuits filed partly by groups opposed to embryo research. Last year, the California Family Bioethics Council and two taxpayer groups argued that CIRM and its board, the Independent Citizens' Oversight Committee (ICOC), are not operating as state agencies because they are not subject to full government oversight. The suit contended, for example, that because ICOC's membership includes scientists from institutions that may apply for grants, they represent their own interests and not those of citizens.

On 21 April, Alameda County Superior Court Judge Bonnie Lewman Sabraw rejected these arguments. CIRM officials and ICOC "are operating in the same fashion as other state agencies," the ruling says. ICOC members have filed financial disclosure forms, the committee has developed conflict-of-interest policies, and it has held public meetings, among other steps. The plaintiffs "have not shown that the Act is clearly, positively, and unmistakably unconstitutional. The Act and the bonds issued thereunder are valid," Sabraw concluded.

"We are extremely pleased," said Robert Klein, chair of ICOC, in a statement. And even though the matter isn't over—appeals could take "at least a year," says CIRM spokesperson Nicole Pagano—the institute is moving ahead, Klein notes. Earlier this month, CIRM issued its first $12.1 million in research training grants, using money raised by selling "bond anticipation notes" (*Science*, 21 April, p. 345). Klein will announce soon another $31 million from the same kind of bonds, Pagano says. (A separate federal lawsuit trying to block CIRM by arguing that fertilized eggs are "persons" was dismissed last year for lack of venue but has been appealed, Pagano says.)

Researchers at California universities in line to receive CIRM funds are rejoicing, too. "We're happy," says Michael Clarke, deputy director of the 4-year-old Stanford Institute for Stem Cell Biology and Regenerative Medicine. He adds, however, that although Stanford has raised other funds to start the institute's work, "its progress is slowed until CIRM is fully functional." **–JOCELYN KAISER**

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PRIZE FOR NEUROBIOLOGY

SCIENTIFIC COMMUNITY

# University Clears Chinese Biophysicist of Misconduct

The leader of a team hailed for the discovery of an antibiotic peptide has been absolved of wrongdoing by his employer. At a press conference last week, Sichuan University in Chengdu, China, announced that allegations of "scientific fabrication" against Qiu Xiao-Qing are unfounded, according to an investigation by a university expert group.

The controversy is unlikely to die down soon, however. The company whose staff leveled the charges has blasted the investigation as lacking "objectivity, fairness, and transparency" and has called on the Chinese government to mount its own inquiry. As *Science* went to press, it was unclear how the government would respond.

In 2003, Qiu, a biophysicist at Sichuan University's West China Hospital, along with 17 co-authors described in *Nature Biotechnology* an engineered peptide with specific antibacterial properties. Chinese media touted the protein, "pheromonicin," or "Ph-SA," as a major breakthrough in antibiotics.

CREDIT: YAO YUAN/*CHENGDU ECONOMIC DAILY*

**Vindicated.** Fraud allegations against Qiu Xiao-Qing are unfounded, a Sichuan University panel says.

Before publication, Qiu applied for a Chinese patent on the peptide and the process of making it. Sichuan NTC Holdings Limited agreed to pay West China Hospital a $250,000 licensing fee; it paid half up front and set up a subsidiary, Chengdu Yanghui Biotechnology, to make pheromonicin. After 2 years of failed attempts at production, Sichuan NTC started to question the patent's validity and refused to pay the second half of the licensing fee. A dispute broke out between Sichuan NTC and West China Hospital, escalating into a fraud allegation. Six of the authors of the 2003 paper wrote to *Nature Biotechnology* last December, alleging that pheromonicin was not "targeted … against specific bacteria" and asking that their names be withdrawn from the paper. Qiu has denied the charge and sued two critics for defamation (*Science*, 17 February, p. 937).

After Sichuan University's news conference, Qiu told *Science* that the names of the six authors-cum-critics were added to the paper when they performed experiments in part to answer questions from *Nature Biotechnology* reviewers. Four authors are Sichuan NTC employees who had been assigned to Qiu's lab to produce pheromonicin for animal safety studies. The other two, of the National Sichuan Institute of Antibiotic Industry, carried out analyses of pheromonicin's antibacterial properties.

In response to the misconduct charge, Sichuan University assembled a panel of experts in microbiology, biochemistry, and molecular biology to conduct experiments to determine whether "the 'falsification' charge … could be substantiated." After 3 months of work, they found "no factual evidence" for falsification, according to a press release. University officials declined to name the panel members or comment further.

Sichuan NTC is not impressed. In a statement, the company called on Sichuan University to release the full investigation report; it says it will refuse to recognize the panel's findings "without a review by government authorities." But it's not clear what agency would handle such an appeal. "China should set up an official mechanism and rules to deal with allegations of academic misconduct," says Yi Rao, a neurobiologist at Northwestern University's Feinberg School of Medicine in Chicago, Illinois.

A separate inquiry has cleared the second corresponding author on the *Nature Biotechnology* paper, George Wu of the University of Connecticut Health Center in Farmington. Spokesperson James Walter says the Health Center's Committee on Research Misconduct found "no credible evidence" to support a misconduct allegation, and therefore "no investigation was conducted." *Nature Biotechnology* is also reviewing the case and will make a decision after Sichuan University relays the investigation results to the journal.

Sichuan University says it will sue those responsible for "irretrievable damage" to its reputation. Qiu, for his part, says the affair has made him loath to get involved in the business end of science: "My place is in the lab." **–HAO XIN**

## Linear Collider Gains Friends

What do an economist, a biologist, and a science policy expert have in common? As members of a recent National Research Council (NRC) committee on particle physics, they all think the United States should spend between $300 million and $500 million total over the next 5 years laying the groundwork for the proposed International Linear Collider (ILC) with the goal of hosting the multibillion-dollar machine. Five years ago, U.S. particle physicists designated the ILC as their future priority, and this week the NRC panel, drawn from various fields, endorsed that vision in a report requested by the Department of Energy (DOE) and the National Science Foundation.

"Not only is the science very exciting, but also if you think in terms of strengthening the physical sciences, then particle physics is an important part of that," says committee chair Harold Shapiro, an economist at Princeton University. Melvyn Shochet, a physicist at the University of Chicago and chair of DOE's High Energy Physics Advisory Panel, says, "I think this report will have legs in Washington more than a report written by particle physicists."

**–ADRIAN CHO**

## Changes in Los Alamos Pensions Trigger Suit

Three unions representing nearly 500 of the roughly 9500 employees of Los Alamos National Laboratory in New Mexico have asked a California state judge to order the weapons lab's new managers to change the pension plan before it goes into effect this summer. The current situation "endangers national security" by pushing out experienced scientists, say the unions.

On 1 June, the new management team, which includes Bechtel and the University of California (UC), will institute a new retirement system that does not include a cash payout and could even force retirees to abandon their UC plans for an as-yet-unannounced retirement fund. UC "threatened and coerced" scientists to accept the changes by tying them to continued employment, according to the suit, which was filed last week.

Arthur Krantz, an attorney for the unions, said the goal is to force UC, which now manages the lab, to make changes in the pension plan before a 15 May deadline for employees to choose among several benefit plans. Last month, DOE's National Nuclear Security Administration said the new benefits were "substantially equivalent," and a lab spokesperson called them fair.

**–ELI KINTISCH**

PLANETARY SCIENCE

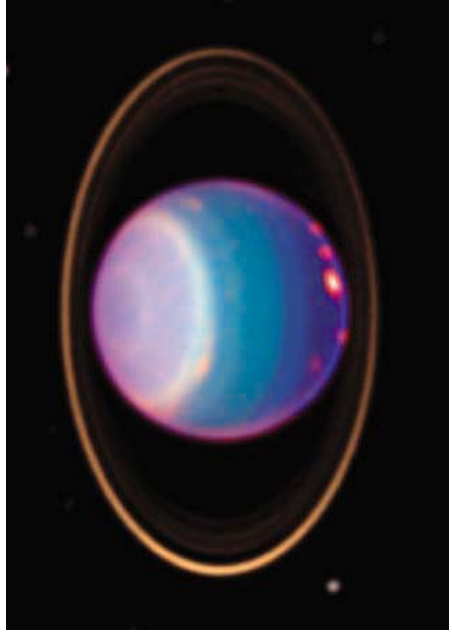
# Simulation Suggests Peaceful Origin For Giant Planet's Weird Spin

How do you knock over a planet? Easy: Just give it a glancing blow from a smaller object. That's how astronomers have always explained the strange fact that Uranus is lying on its side, with its spin axis almost parallel to its orbit around the sun. But an Argentine astronomer says violence is unnecessary: Uranus's axial tilt, along with the tilts of its fellow gas giant planets, can be explained by gravitational perturbations alone.

For decades, astronomers have invoked giant impacts in the chaotic aftermath of the solar system's birth to account for the origin of the moon, the thin, rocky mantle of Mercury, and the formation of the rings of Saturn. Little wonder that they also thought a tangential collision could tip over a planet. In the case of Uranus, however, the collision scenario has one important downside: A sudden cosmic smash would have left its moons unscathed. Yet the orbits of Uranus's regular satellites are also tipped over—they still circle the planet in its equatorial plane. So any tilting collision must have happened during a very early, brief stage, when the planet was still enveloped in a thick disk of material from which the satellites would later condense. "The idea always seemed a little improbable to me," says planetary dynamicist Scott Tremaine of Princeton University.

Adrián Brunini of the National University of La Plata, Argentina, agrees. In this week's issue of *Nature*, Brunini presents computer simulations that show how the obliquities of the giant planets arise naturally from mutual gravitational perturbations. These were strong in the early history of the solar system, when the young planets were slowly changing orbits owing to their interaction with the remaining rubble in the solar nebula.

Brunini started off with a migration scenario that has been shown to provide the best explanation for the current orbital layout of the outer solar system. In most of his simulations, Uranus ends up on its side, Saturn and Neptune achieve a reasonable tilt, and Jupiter stays almost upright, exactly as observed. The strong tilt of Uranus results from close encounters with Saturn that occurred at a time when an orbital resonance between Jupiter and Saturn greatly increased the eccentricities of the more distant giant planets. ▶

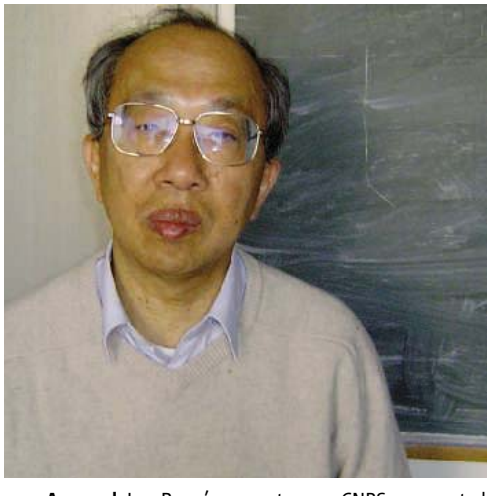


**Sideways.** Uranus's equator and the orbits of its rings and moons lie 98° from its orbital plane.

FRANCE

# Chemist Claims Innocence to Spying Charge

**STRASBOURG, FRANCE—**It started out like a spy movie. On the morning of Saturday, 8 April, a border police officer at the airport here found four vials containing a white substance in chemist Luu Bang's suitcase. Instead of flying to Paris, where he was supposed to catch a connection to China, Luu found himself arrested and questioned; his lab and home office were searched; and his employer, the National Center for Scientific Research (CNRS) filed a theft report with the police.



**Accused.** Luu Bang's supporters say CNRS overreacted to Luu's failure to fill out paperwork.

Luu, 66, was released after 10 hours. But more than 2 weeks later, his career and reputation are still on the line. A judicial inquiry is ongoing, CNRS has sent him into immediate retirement, and he has had to defend himself against espionage charges in the press. Yet his only mistake, he says, was not filling out the proper paperwork for the vials, which contained well-known chemicals developed in his lab. "This has hit me like a meteorite," he says. Colleagues, too, insist CNRS has overreacted to a common infraction.

Born in Phnom Penh, Cambodia, to a family of Chinese origin, Luu came to France to study at age 18. He has worked at the CNRS Institute for Neurochemistry in Strasbourg for more than 35 years; his work currently focuses on compounds that can activate stem cells. The French consulate in Guangzhou, in southern China, had invited him to visit this month—and was paying his expenses—to foster Sino-French collaboration. Luu says consular officials knew he was bringing the vials, containing long-chain fatty alcohols, so that prospective Chinese partners could try them in their own labs. Although he admits he erred by not obtaining authorization first, Luu says the notion that he committed theft or espionage is absurd. The compounds have been described in the scientific literature and are patented by CNRS both in Europe and China, he says.

But Philippe Piéri, who heads CNRS's Strasbourg office, says it was a "grave mistake" not to get permission to export the vials. "Scientists can't just do what they think is right, like in the 19th century," he says. Luu has been retired 4 months ahead of the scheduled date, and he won't be allowed to work as an emeritus, Piéri says.

The prosecutor's office in Strasbourg declined to comment on its investigation; Luu says he thinks it started petering out once it became clear that he had been invited to Guangzhou by French diplomats. But Luu, a French citizen who feels his loyalty was questioned because of his ethnic background, wants CNRS to retract its sanctions too.

Luu's main defender is his Ph.D. supervisor Guy Ourisson, 80, a former president of the French Academy of Sciences. In a 20 April letter to CNRS Director General Arnold Migus, co-signed by six Strasbourg chemists including one Nobelist, Ourisson called the penalty "entirely out of proportion" and asked that it be lifted. Ourisson says he and others have unknowingly violated the same rules "dozens of times." In Luu's case, he says, "CNRS seems to have acted on the general fear that China is out to rob us." **–MARTIN ENSERINK**

CREDITS (TOP TO BOTTOM): JPL/NASA; DJAUL LOOWAR

Tremaine says Brunini's results provide "active support for the idea that substantial migration has indeed occurred." Theoretical astronomer Jack Lissauer of NASA's Ames Research Center in Moffett Field, California, says he is surprised by the very narrow range of resulting obliquity values in Brunini's computer runs: "It's very interesting to see how precisely his results agree with the actual values."

But although there's no reason anymore to believe that Uranus was knocked over by a planetary collision, Lissauer says such events can't be ruled out altogether. "There still could have been big things flying around to do the hits," he says, "even before the formation of the satellite systems." **–GOVERT SCHILLING**

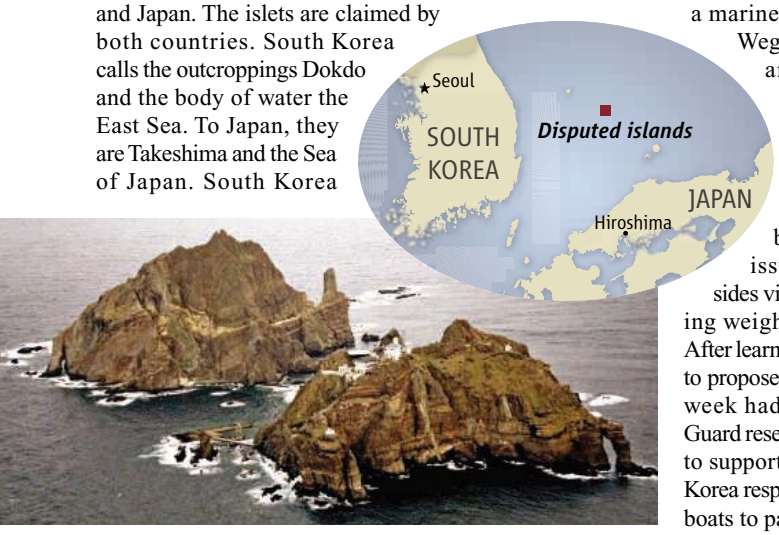
Govert Schilling is an astronomy writer in Amersfoort, the Netherlands.

OCEANOGRAPHY

# Korea and Japan Clash Over Surveys

**TOKYO**—A little-known international agency that approves the names of sea-floor topographic features found itself caught in the middle of a high-stakes territorial spat last week between Japan and South Korea. The two countries have backed away from a confrontation over dueling surveys in disputed waters, at least for the moment. But the fate of survey data—and of the coveted territory itself—still hangs in the balance.

The dispute centers on a cluster of islets, and the surrounding exclusive economic zone (EEZ), roughly halfway between South Korea and Japan. The islets are claimed by both countries. South Korea calls the outcroppings Dokdo and the body of water the East Sea. To Japan, they are Takeshima and the Sea of Japan. South Korea controls the islets thanks to a police garrison on one of the rocks. The nations are vying for rights to exploit fishing grounds and extract what may be substantial offshore deposits of methane hydrates.




**A rock by any other name.** Korea and Japan are vying to exploit the natural resources off these isolated islets.

PHOTO CREDIT: KWON JOO-HOON/AFP/GETTY IMAGES

Partly because of the contretemps, the sea floor near the islets had not been surveyed since a Japanese-led effort in the 1970s—that is, until a South Korean expedition last year. South Korea's hydrographic survey "found many new [subsea] features," including seamounts and troughs, says Seok-Chang Kwon, head of the Marine Research and Development Division of South Korea's Ministry of Maritime Affairs and Fisheries. "It's our right to name the features we found," he says. The ministry was planning to propose Korean names for consideration at a 21 June meeting of the Sub-Committee on Undersea Feature Names of the General Bathymetric Chart of the Oceans, an organization that standardizes and publishes nautical information under the auspices of the Monaco-based International Hydrographic Organization (IHO) and UNESCO's Intergovernmental Oceanographic Commission. Naming a feature is "in general, first-come, first-served," as long as there are good supporting survey data, says subcommittee chair Hans-Werner Schenke, a marine geologist at the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven, Germany.

Officials in South Korea and Japan agree that the names of subsea features and the EEZ boundaries are separate issues. Nonetheless, both sides view the name game as giving weight to competing claims. After learning of South Korea's plans to propose names to IHO, Japan last week had dispatched two Coast Guard research vessels to gather data to support Japanese names. South Korea responded by sending 20 gunboats to patrol the disputed waters. Two days of tense negotiations yielded a compromise: Japan canceled its survey, and South Korea pledged to postpone proposing names. And the countries agreed to resume stalled talks on the EEZ boundaries.

No matter the outcome, "the committee encourages the exchange of new survey data," says member Lisa Taylor, a geophysicist with the U.S. National Oceanic and Atmospheric Administration in Boulder, Colorado. (Korea's National Oceanographic Research Institute has posted survey data on its Web site.) The information is useful not only for navigation, Taylor says, but increasingly for geographical, geological, and paleontological research as well. **–DENNIS NORMILE**

## NYU Gift Kicks Up More Dust

A prominent Harvard archaeologist is rallying support for working with unprovenanced artifacts, following a controversy surrounding the recent $200 million gift to New York University (NYU) from the Leon Levy Foundation (*Science*, 31 March, p. 1846). Lawrence Stager, whose excavations at Ashkelon, Israel, are funded by the foundation, argues in a "Statement of Concern" that "unprovenanced" artifacts should be the legitimate object of study. More than 100 archaeologists and historians have signed the statement (www.bibarch.org/bswbOOunprovenanced.html).

The 11-point statement criticizes the policies of the Archaeological Institute of America (AIA) and other organizations that prohibit the first publication of unprovenanced antiquities in their journals as well as presentations of such objects at their meetings. "The antiquities market is often the means by which [unprovenanced objects] are rescued," the statement says, citing the Dead Sea Scrolls and the Gospel of Judas as examples.

AIA President Jane Waldbaum says the statement mischaracterizes the organization's policies. "At no time was any attempt made to … prevent the scholarly discussion of archaeological objects," Waldbaum writes on the association's Web site. The goal is to avoid promoting artifacts with questionable provenance.

**–MICHAEL BALTER**

## Scripps Florida Deal in Jeopardy

It's down to the wire again for Scripps Florida, the East Coast offshoot of the La Jolla, California–based research behemoth. A 2 May deadline looms to resolve an impasse with Palm Beach County officials over the opening of a Scripps branch in Jupiter. At issue: jobs. Scripps officials hope to create 545 jobs in return for $369 million from Florida and about $200 million from the county. County officials say the institute should be liable if it fails to produce the promised number of jobs or leaves before the 30-year deal is up.

This week, County Commissioner Burt Aaronson offered Scripps a compromise: Take out a $100 million bond, and the county will knock 15 years off the deal. But Scripps officials balked at the expense, saying the bond would require collateral and cost about $23 million in premiums. "We've said all along [the deal] can't put any of our assets in La Jolla at risk," says Scripps spokesperson Keith McKeown.

But don't count the deal out just yet. Scripps officials badly want to open a new Florida facility next year. And that would be hard to pull off at another site if the current deal falls through.

**–ROBERT F. SERVICE**

GENETICS

# Parasite-Resistant Mosquitoes: A Natural Weapon Against Malaria?

In a world where mosquitoes were resistant to infection with parasites, no human being would suffer from malaria. With that idea in mind, some researchers are trying to sneak resistance genes into mosquitoes and encourage those genes to spread through the population.

But a paper on page 577 of this issue suggests that engineering resistance into mosquitoes may be unnecessary. In an endemic area in Mali, researchers found that many *Anopheles gambiae* mosquitoes—Africa's most important malaria vector—are already resistant to *Plasmodium falciparum*, the malaria parasite. Resistance appears to reside in one or more genes in a very small genomic region, the researchers found—and the mosquitoes that don't have those genes may just be the odd ones out.

**Filling up.** Mosquitoes feed on malaria-infected human blood through a membrane. To researchers' surprise, many are resistant to the *Plasmodium* parasite.

The surprising upshot, according to Matt Thomas of the Commonwealth Scientific and Industrial Research Organisation in Canberra, Australia: "Why put new resistance genes into mosquitoes if they already have their own?" Instead, maybe the goal should be to eliminate the minority population that's susceptible, Thomas says. Its implications aside, the study's combination of fieldwork and molecular genetics is "most wonderful," says Sergey Nuzhdin of the University of California, Davis. "I'm very envious."

In the study, researchers from the University of Minnesota, the Fred Hutchinson Cancer Research Center in Seattle, Washington, Princeton University, and the University of Bamako in Mali set out to find genes that determine malaria resistance in nature, using a tried-and-true strategy: Look for variation in a trait within families, and then use genetic markers to discover where the corresponding genes are located.

The group collected female mosquitoes inside huts in Mali and let each produce one generation of offspring. Then, they let the resulting pedigrees feed on blood from a malaria-infected villager; after 7 to 8 days, they sliced open the insects and counted the oocysts—a stage in *Plasmodium*'s life cycle—inside the insect gut. The lower the number, the more resistant the individual.

They discovered that a small region on the 2L chromosome of *A. gambiae* played an all-important role. The *Plasmodium* Resistance Island, as they dubbed it, contains almost 1000 genes. Using several techniques to shake out genes of relevance, they pinpointed one gene, *APL1*, that appears to play a particularly important role; when its action was blocked using RNA interference, mosquitoes became vulnerable to infection. Still, other nearby genes may be involved as well, says lead author Kenneth Vernick of the University of Minnesota, St. Paul.

What surprised the team most was how widespread resistance is; in 22 of 101 pedigrees, not a single insect became infected after supping on infected blood. Like many other researchers, Anthony James of the University of California, Irvine, assumed that most mosquitoes were naturally susceptible to malaria infection—"until I read this paper," he says. James leads a consortium that has received almost $20 million from the Bill and Melinda Gates Foundation to develop a dengue-resistant mosquito; his group is working on malaria-resistant counterparts as well. "It's very interesting to think we're really targeting a much smaller part of the population than we thought," he says.

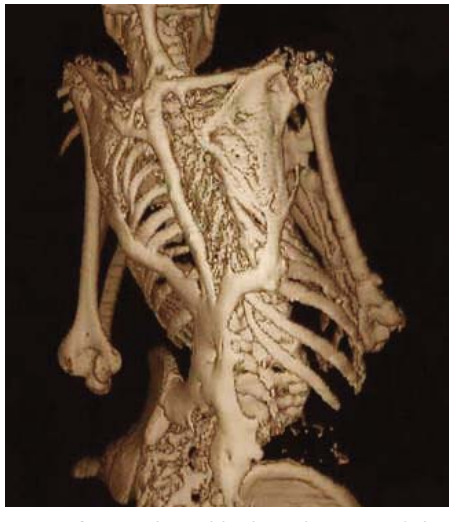
But Vernick goes a step further: Instead of introducing new genes, why not try to wipe out the minority susceptibility alleles? One possible strategy, he says, would use insect-devouring fungi that two studies identified as potential weapons against malaria last year (*Science*, 10 June 2005, p. 1531). The work suggested that the fungi preferentially kill *Plasmodium*-infected mosquitoes. If that's true, spraying with the fungi might drive susceptibility genes out of existence, Vernick says.

Willem Takken of Wageningen University in the Netherlands, who co-authored one of the papers, says that "it may be a bit utopian, but it's a very interesting idea."**–MARTIN ENSERINK**

BIOMEDICAL RESEARCH

# Bone Disease Gene Finally Found

Before dozens of people in an auditorium at the University of Pennsylvania, announcing the biggest discovery of his career, Fred Kaplan fought back tears. His 15-year search for the gene behind a rare and horrifying bone disease had ended, fingering a single DNA base as the culprit and offering hope to the small number of people afflicted with the often fatal illness. Three days before, Kaplan, an orthopedic surgeon, had privately shared the news that the gene search was over with some members of the International Fibrodysplasia Ossificans Progressiva (FOP) Association. "We were all crying," he says.



**Trapped.** Extra bone blankets the torso of this 12-year-old who has a genetic disease in which sufferers grow a "second skeleton."

The relentless hunt for the FOP gene had tightly bound Kaplan and a small band of researchers to FOP families from places as far away as the Amazon rainforest, rural Georgia, Bavaria, and South Korea. Thanks to fundraising efforts such as barn dances in Scotland and sales of barbecued chickens in California, these families' communities have collected about 75% of the money used in FOP research.

In people with FOP—2500 or so are thought to be living with the disease—muscle and connective tissue gradually turn to apparently healthy bone, freezing the neck, spine, hips, and even jaw into place and trapping patients inside a "second skeleton." The newly discovered gene mutation, described this week online in *Nature Genetics*, not only has potential therapeutic implications for the currently untreatable disorder, but it may also reveal novel avenues for harnessing the tragic talent

CREDITS (TOP TO BOTTOM): COURTESY OF K. VERNICK; CREDIT: E. SHORE AND F. KAPLAN/UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE; *NATURE GENETICS* (23 APRIL 2006); DOI: 10.1038/NG1783

of FOP patients to produce prolific amounts of bone. "We always need hard tissue," says Patrick Warnke of the University of Kiel in Germany, who is exploring ways to grow bone and performs facial reconstruction on patients who have lost bone to cancer or trauma. The FOP gene defect, he says, could "show us the way to induce bone growth."

The FOP defect appears in a gene, called *ACVR1*, that lies along a well-known pathway that controls the formation of bone and cartilage. Kaplan, University of Pennsylvania geneticist Eileen Shore, and their colleagues discovered in the 1990s that FOP patients had defects in this pathway, but they couldn't identify the underlying gene mutation until recently. The mutant form of *ACVR1* found in people with FOP produces a protein that has an altered amino acid sequence and is possibly overactive; the normal ACVR1 protein seems to signal cells to boost production of a so-called bone morphogenic protein that spurs bone growth and to clamp down on other proteins that inhibit bone proliferation.

The gene, says Michael Longaker, a craniofacial surgeon at Stanford University in California, offers "an accelerator and a brake" to bone growth. In people needing new bone, boosting *ACVR1*'s expression locally could be a way to induce their bodies to grow some on their own. In FOP patients, blocking the receptor with a drug or perhaps a targeted therapy such as RNA interference could retard or prevent the condition's uncontrolled bone growth.

The search for the FOP gene was marred by wrong turns. In 2000, Kaplan's team published a paper linking FOP to chromosome 4, then failed to find the same pattern in additional patients. (*ACVR1* is on chromosome 2.) The previous year, a French group claimed to have identified a candidate gene, but its results weren't replicated.

Faced with an uncommon disease in which families with more than one FOP member are vanishingly rare—few sufferers have children, and most develop the disease because of a random mutation—Kaplan issued an "all points bulletin" to doctors worldwide to send families his way. In the end, just five families with multiple members with FOP provided the critical DNA needed to identify the *ACVR1* mutation. The single-nucleotide variation identified in them has been found in all 50 FOP patients tested and is absent from all of 159 controls.

"That it's so specific is pretty amazing," says Harvard University geneticist William Gelbart. He hopes that an FOP mouse model can now be created, allowing for deeper study of the disease and potentially drug development, something Kaplan, Shore, and others are already working on.

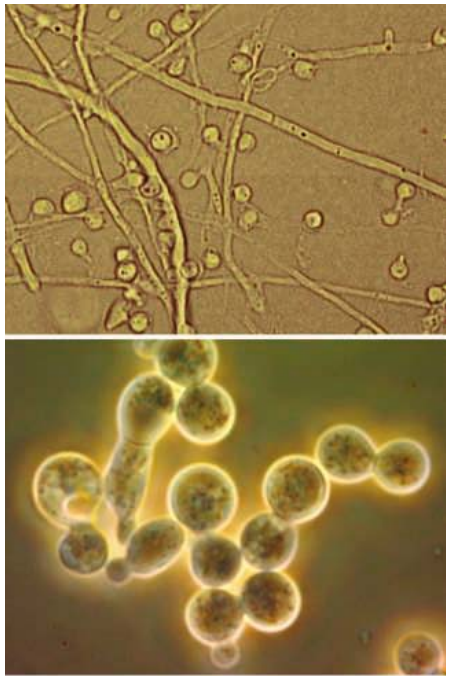
Kaplan acknowledges that an FOP treatment may still be many years away. For now, his overriding emotion, after such a prolonged gene search, is relief. **–JENNIFER COUZIN**



MICROBIOLOGY

# Environmentally Sensitive Protein Proves Key to Making Yeast Pathogenic

A handful of common soil molds are the Jekylls and Hydes of the fungal kingdom: Human body heat triggers their transformation from a benign fungus to a pathogenic yeast. On page 583, Bruce Klein, an infectious diseases physician at the University of Wisconsin, Madison, and his colleagues reveal a single gene that sets in motion this pernicious makeover.



**Good guy, bad guy.** Body heat can turn some soil molds (*top*) into pathogenic yeast (*bottom*).

"These fungal pathogens have been extremely difficult to study," says Joseph Heitman, a molecular medical mycologist at Duke University in Durham, North Carolina. "This is a terrific paper." And because the human version of this triggering gene is not functional, it could be a useful target for drugs to treat these infections, says Klein.

Six species of soil molds are known to undergo the Jekyll-to-Hyde transformation, causing coughs, fevers, and other symptoms when inhaled. The organisms change from a form that reproduces through spores to one that reproduces by budding—a much more efficient process in humans.

Researchers already knew that a fungal gene called *BAD1* helps the fungus stick to the lung's lining, move into the lung cells, and avoid destruction by the immune system. But no one could figure out what turned on this gene. "This is one of the big questions that has captured my imagination and that of this field for many years," says Klein.

To answer it, Klein's graduate student Julie Nemecek made 15,000 mutants of *Blastomyces dermatitidis*, one of the Jekyll-and-Hyde fungi. She exploited a bacterium previously used in plant genetic engineering to insert gene-disrupting pieces of DNA at random places in the fungus's genome. She applied this technique to *B. dermatitidis* strains that had already been engineered to turn blue when the *BAD1* gene was fully activated and white when it wasn't.

From the 15,000 mutants, Nemecek identified seven white cultures, one of which had almost no signs of *BAD1* activity. The shape of that fungus, as well as the composition and structure of its cell walls, were not very yeast-like, and the number of infectious spores the defective fungus produced in artificially warmed conditions shrank by 90%.

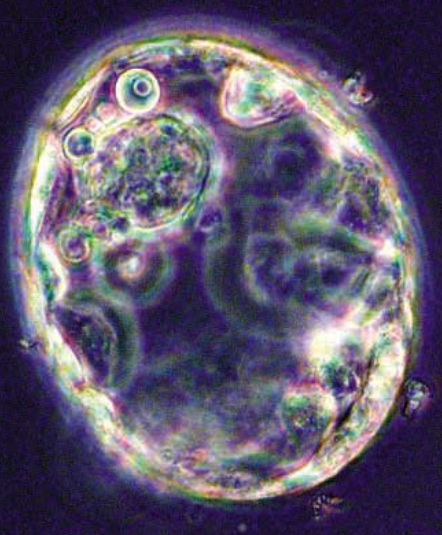
Nemecek then identified the gene that was disrupted in this mutant strain. She and Klein concluded that, based on its DNA sequence, the gene codes for a protein called histidine kinase. This enzyme and its relatives help organisms sense changes in their environments, including temperature shifts. They are ancient, existing throughout the tree of life.

The researchers named the gene *DRK1* for dimorphism-regulating histidine kinase. When Nemecek specifically knocked out this gene in *B. dermatitidis*, or dampened its activity using a method called RNA interference (RNAi), the fungus produced few to no spores. And when she used RNAi on another Jekyll-and-Hyde fungus, the mold only poorly converted into yeast when warmed, suggesting that *DRK1* might be key in all six pathogenic species. "This is an excellent piece of work in finding the key regulator of fungal dimorphism," says K. J. Kwon-Chung, a molecular mycologist at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland, which funded the work.

Marcel Wüthrich, an immunologist at the University of Wisconsin Medical School in Madison, also exposed mice to spores with defective *DRK1*. The lung infections were much less severe than when the mice were infected with unaltered molds.

Other researchers have discovered that histidine kinases exist in bacterial pathogens, suggesting that they control virulence in many microbes. If so, these enzymes may "serve as a global target for drug discovery," says Richard Calderone, a medical mycologist at Georgetown University in Washington, D.C.

**–ELIZABETH PENNISI**



**Several groups around the world are trying to do what Woo Suk Hwang fraudulently claimed to have done**

# Picking Up the Pieces After Hwang

**A YEAR AGO, IT SEEMED SO EASY. IN MAY** 2005, Woo Suk Hwang and his colleagues told the world that they could make embryonic stem (ES) cells from cloned human embryos with an efficiency that astounded—and thrilled—their colleagues. In roughly one out of every 12 tries, the South Korean team reported, they could produce ES cell lines that were a genetic match to patients. Scientists hoped to use such cells to probe the genetic triggers of diseases such as diabetes and amyotrophic lateral sclerosis (ALS). Some dreamed of using them as the raw material for developing new tissues and cells that could treat previously incurable maladies.

A few months ago, those claims famously unraveled. It is now clear that Hwang's team does not have any ES cell lines created from patients. It is also clear that the group didn't fail for lack of trying: The team apparently used more than 2200 donated human oocytes in their experiments—more than five times the number they claimed in their papers (*Science*, 10 February, p. 754). The meltdown dashed the hopes of researchers and patients around the world, leaving many wondering whether cloning might be too difficult after all.

But as the shock of the scandal wears off, a handful of groups around the world are trying to do what Hwang and his group apparently couldn't. At least three groups in the United States, three in Europe, and one in China say they are preparing to start efforts to derive ES cells from cloned human embryos. In attempting this feat, they all face two substantial hurdles: a limited supply of human oocytes and a lack of data on how to use them most efficiently.

Most researchers agree that they have to discount nearly everything they thought they had learned from Hwang, but they also know that Hwang's techniques did achieve some successes. The lab does have one confirmed—and unprecedented—claim: It cloned a dog. And investigators at Seoul National University concluded that the lab did produce cloned human blastocysts, or week-old embryos, in about one out of every 10 attempts. But the team apparently failed to derive viable ES cells from those cloned embryos. It is not clear whether the fault lies with low-quality embryos generated by cloning or with the techniques the team used to try to derive stem cells.

A collaboration at Harvard Stem Cell Institute is set to find out. Even before Hwang's claims fell apart, researchers there were planning to try their hands at deriving human ES cells through a process known as somatic cell nuclear transfer (SCNT). A successful derivation involves two distinct steps, both of which require considerable skill. In SCNT, scientists remove the nuclear DNA from an oocyte, attempting to inflict as little damage on the cell as possible. They then fuse the enucleated oocyte with a skin cell or other somatic cell. The oocyte provides signals that reprogram the somatic cell DNA and enable it to direct the development of an early-stage embryo. To make ES cell lines, scientists next isolate the group of cells called the inner cell mass from week-old cloned embryos and coax them to grow in culture dishes.

Now, almost 2 years after they started, Douglas Melton and Kevin Eggan of Harvard University and George Daley of Harvard Medical School in Boston have accumulated nearly all the approvals and permissions they need to start accepting oocyte donations. The process has involved at least five ethics committees and Institutional Review Boards, which must review the ethical safeguards governing donations of oocytes and also of somatic cells from patients. Because current government rules prohibit the use of federal money to derive new human ES cell lines, the Harvard team is funding this effort—including the facilities—with money from the Stowers Medical Institute in Cambridge, Massachusetts, the Juvenile Diabetes Research Foundation International in New York City, and other private donors.

The Harvard team wants to create cell lines from patients with diabetes and ALS, which they hope will help researchers understand the genetic and

**After the fall.** Woo Suk Hwang and his colleagues do not have the stem cells they claimed to have made from cloned human blastocysts.

CREDITS (TOP TO BOTTOM): MIODRAG STOJKOVIC; CHUNG SUNG-JUN/GETTY IMAGES

**Best yet.** Miodrag Stojkovic and Alison Murdoch and their colleagues generated this cloned human blastocyst but were not able to derive ES cells from it.

molecular processes that drive these diseases. The group will rely on so-called compassionate donors, women who are willing to donate oocytes specifically for research. Eggan and his colleagues hope that using fresher, healthier oocytes than those left over after in vitro fertilization (IVF) procedures will increase the chances of success. Hwang and his colleagues reported that freshly harvested oocytes from women younger than 30 were significantly more efficient than oocytes from women 30 or older. That claim is plausible in light of well-established fertility statistics, say researchers, but can't be completely trusted. Harvard researchers have said they hope to attract women younger than 30 as donors.

Two other U.S. groups, in New York City and San Francisco, say that for their first efforts they will rely on excess oocytes from women undergoing fertility treatments. One of the team leaders, fertility expert and developmental biologist Renee Reijo-Pera of the University of California, San Francisco (UCSF), had planned to send students to Seoul to learn Hwang's techniques. With those plans scotched, the team has a protocol under review at the university that would use oocytes collected for IVF treatments but which failed to fertilize in the culture dish. Such oocytes are likely to be lower quality, but they would otherwise be discarded, so the ethical questions surrounding their use are less troubling. "We are still at a stage where the technology [for human SCNT] has not been properly developed," says Arnold Kriegstein, director of UCSF's stem cell biology program. Until researchers know more about which techniques might work best, he says, they will avoid treating volunteers with the ovary-stimulating drugs required for egg donation, which can cause serious complications. The work is being funded by private donations.

The lab of developmental biologist Lorenz Studer at Memorial Sloan-Kettering Cancer Center in New York City was one of a handful that was working with several cell lines from Hwang's lab when the scandal broke. Investigators later determined that the lines were most likely not created through cloning but arose either from early parthenogenetic development, in which an unfertilized oocyte begins dividing, or from IVF-derived embryos. Studer, who says he has not heard from Hwang since fraud allegations were first raised, will now collaborate with colleagues at Rockefeller University and Weill Cornell Medical Center. The three institutions received a $50 million grant from the Starr Foundation in New York City last year to focus on stem cell research, part of which will fund nuclear transfer to create cell lines from ALS and Parkinson's patients.

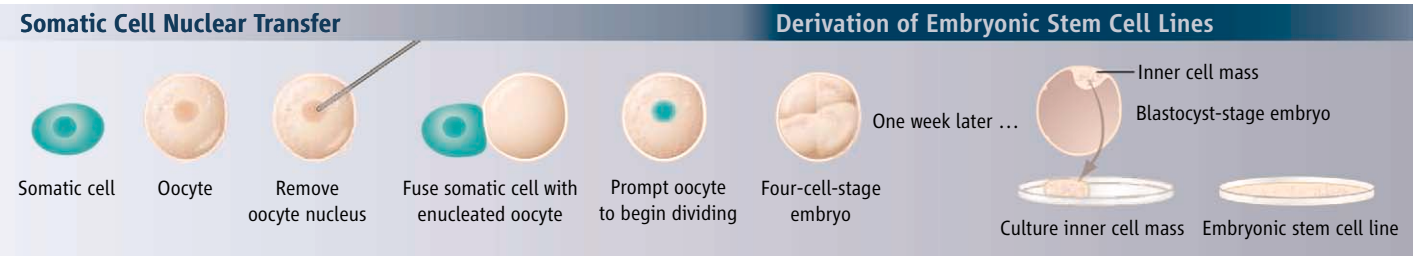
Studer cautions, however, that successful cloning attempts may be few and far between. "I don't doubt that you can do it, but the efficiency might be so low that you couldn't do it on a practical level," says Studer, who hopes to use ES cell lines for both basic research and drug screening. "It looks like the most likely efficiency is 10 times lower than [Hwang and his team] claimed" last year—which might mean a success rate of one out of more than 200 tries.

In Europe, at least three groups have said publicly that they hope to get human cloning working in their labs. All are being funded at least in part by government grants. A group led by Ian Wilmut of the University of Edinburgh and Christopher Shaw of King's College London received a license from Britain's Human Fertilisation and Embryo Authority in February 2005 to conduct human nuclear transfer experiments, but Wilmut says the scandal has prompted them to rethink their plans: "It was necessary to spend some time unlearning some things that we thought we had learned from Hwang's research." The researchers are now preparing a new application for permission and funding for a slightly different approach to creating ES cell lines from Parkinson's and ALS patients, he says. The researchers may attempt to use rabbit instead of human oocytes, he says. (Researchers in China have reported deriving human ES cell lines from embryos generated through SCNT using rabbit oocytes.)

After the Hwang debacle, researchers at the University of Newcastle upon Tyne in the United Kingdom hold the distinction of having published the only paper on human cloning that has not been discredited. Alison Murdoch, Miodrag Stojkovic, and their colleagues reported in 2005 in *Reproductive Biomedicine Online* that they were able to create a single human blastocyst, although they could not derive ES cells from it. Murdoch declines to discuss recent progress until the team is ready to publish another paper.

Stojkovic has since moved to Valencia, Spain, where he is deputy director at the Prince Felipe Research Centre, a $180 million facility funded by local and national governments and private sources. In March, the Spanish government legalized human nuclear transfer experiments; Stojkovic is now seeking approval from a national ethics committee. He says his team could start working with human material as early as this summer.

Stojkovic says he will obtain oocytes from a large fertility hospital in Valencia that manages 3000 cycles of fertility treatment per year. But he says he won't bother with leftover oocytes that failed to fertilize in the lab: "From what I have seen, the potential [of fail-to-fertilize oocytes] is equal to zero. We need fresh human eggs. What you get left over from the IVF clinic is not viable." In fact, he says, every minute counts. In the paper describing the cloned blastocyst, he and his colleagues reported that oocytes were most effective if they were enucleated within an hour after collection. He says he hopes to find women who produce significantly more oocytes than they need or who would be willing to donate some of their oocytes in exchange for a discount on the cost of their fertility treatment.




Finally, a team at the Chinese Academy of Sciences' Shanghai Institutes for Biological Sciences is now seeking approval for human cloning. "Hwang's work was fake, but someone has to do the real thing," says Guotong Xu, deputy director of the Institute of Health Sciences there. The stumbling block is not likely to be approval, says Xu, but money, as no one knows whether China's funding agencies consider human SCNT efforts worthwhile.

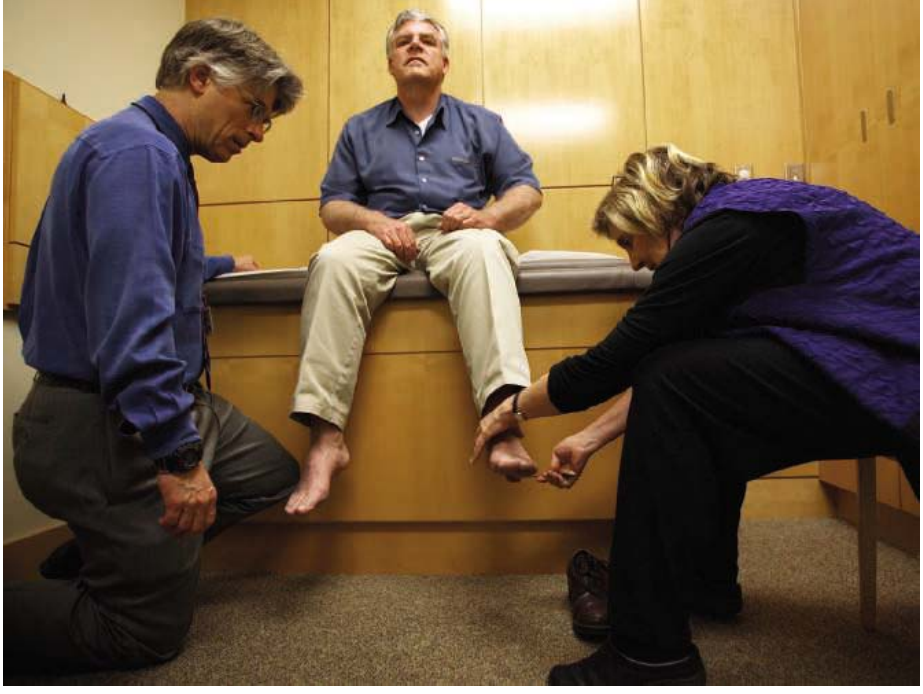
As the field attempts to rebuild post-Hwang, Studer hopes the groups will behave like informal collaborators rather than rivals. "It is important that we all stay in contact … so we know what we are each trying to do," he says. Oocytes are scarce enough that teams should try to waste as few as possible—and should avoid directly duplicating each other's work, he says.

Stojkovic says he is optimistic that someone will soon succeed where Hwang and his colleagues failed. "I have no doubt that soon someone will have cloned human stem cells," he says. "I don't know any technical, biological, or ethical reasons we should not continue."

**–GRETCHEN VOGEL**

With reporting by Dennis Normile.

ILLUSTRATION: P. HUEY/*SCIENCE*



**Checkup time.** Paul and Randi Hagerman examine an FXTAS patient who is the grandfather of a child with Fragile X.

BIOMEDICAL RESEARCH

# Fragile X's Unwelcome Relative

**By studying the grandfathers of children with Fragile X syndrome, scientists have found a surprisingly common neurological disorder that may be due to abnormal RNA**

Randi Hagerman may be the only pediatrician to discover a disease that strikes in old age. Hagerman specializes in treating children with Fragile X syndrome, the most common inherited form of mental retardation. Several years ago, she began to notice something odd when she chatted with her patients' parents. "Typically, the moms would bring the children in to see Randi, and in the course of the discussion, the moms would say, 'I'm concerned about my father. He's falling down a lot,' " says molecular biologist Paul Hagerman, Randi's husband and research collaborator. "This was a pattern she would hear over and over."

At first, the Hagermans suspected this was nothing more than a few isolated cases of ataxia, or coordination problems. That changed in 2000, when Randi presented neurological workups of a small group of her patients' grandfathers at a Fragile X conference for researchers and parents. At the end of her talk, she asked if anyone in the audience had seen similar problems. "Of the mothers in the room, I would say a third of the hands went up," Randi says. "It was an epiphany of sorts," Paul recalls.

Follow-up studies by the Hagermans, now at the University of California (UC), Davis, and collaborators have recently documented a suite of symptoms that strike the relatives—most often the maternal grandfathers—of children with Fragile X. These men are typically healthy early in life and have average to above-average IQ's. But in their 50s and 60s, many begin to experience tremors and movement difficulties that grow progressively worse. Studies have turned up cognitive and psychiatric problems in these men as well. The symptoms are far more disabling than the general decline people experience with age, and they can lead to death.

The newly identified disorder, called Fragile X–associated tremor/ataxia syndrome (FXTAS), may turn out to be one of the most common inherited forms of neurodegenerative disease. Work by the Hagermans and others has linked FXTAS to the same gene responsible for Fragile X—even though the two disorders are drastically different. Researchers are now studying postmortem brain tissue from FXTAS patients and creating genetically altered fruit flies and mice in hopes of unraveling the disorder's underlying biology. Physicians are also documenting the clinical progression of FXTAS, work that should help neurologists avoid misdiagnosing it—as happens often.

"At first, no one was quite sure this was real," says Stephen Warren, a geneticist at Emory University in Atlanta, Georgia, and a co-discoverer of the genetic mutation that causes Fragile X. Doctors had always told relatives of children with Fragile X syndrome that they had no reason to expect health problems themselves and that their only risk was passing on a bad gene to the next generation. Now, says Warren, it's clear that this counsel was misguided.

### A puzzling premutation

Fragile X syndrome earned its name from the brittle appearance of the X chromosome in people with the disorder: Under a microscope, part of the chromosome looks as if it's dangling by a thread. In 1991, researchers identified a mutated gene that resides in that part of the chromosome. A genetic stutter gives the gene, called *FMR1*, 200 or more repeats of the same sequence of three nucleotides: a cytosine followed by two guanines, or CGG. People without Fragile X have about 30 CGG repeats in *FMR1*, but 200-plus repeats disables the gene, and its protein, called FMRP, doesn't get made. How the lack of FMRP causes mental retardation and other Fragile X symptoms isn't clear, but researchers have recently gotten excited about a theory linking the deficit to aberrations of neural plasticity (see sidebar, p. 521).

In some ways, the inheritance pattern of Fragile X sticks to the script every student learns in Genetics 101. Because a boy's X chromosome always comes from his mother, he can only get a bad *FMR1* gene from mom. And because they have only one X chromosome, boys who inherit the Fragile X mutation have no other way to make FMRP. But girls are complicated. Despite having a backup copy of *FMR1* on their second X chromosome, girls can also develop Fragile X, although they tend to have less mental retardation.

Another puzzle about the genetics is that most mothers of Fragile X sons have fewer than 200 CGG repeats themselves. Instead, they carry a "premutation" with an intermediate number of repeats ranging from 55 to 200. Through some still-mysterious process, the number of repeats expands into the full mutation that causes Fragile X when passed from mother to offspring. Men can also carry a premutation and pass it on to their daughters. (Only daughters inherit dad's X chromosome.)

Back in 1999, when Randi Hagerman started growing concerned about the maternal grandfathers of her patients, she consulted neurologist Maureen Leehey, a movement specialist at the University of Colorado Medical Center in Denver, where the Hagermans worked at the time. Several of the men had been told they had Parkinson's disease, which involves degeneration of the basal ganglia, a brain region that helps execute movements. But Leehey's neurological tests pointed to problems in a different brain region. The men did poorly, for example, on a test called the tandem gait test—the toe-to-heel walk police use to assess the sobriety of suspected drunk drivers. Parkinson's patients do surprisingly well on this test, Leehey says, but the grandfathers could barely stand with one foot in front of the other, let alone walk in a straight line. That suggested a problem in the cerebellum, a structure at the back of the brain that's important for balance and coordination.

CREDIT: JOHN LEE/GETTY IMAGES

Subsequent brain scan studies have confirmed this hunch, revealing shrinkage in the middle cerebellar peduncle, a major communication link between the cerebellum and brain stem. These studies have also found signs throughout the brain of degenerated white matter, the axons carrying signals from neuron to neuron.

What could cause axons to wither? The *FMR1* gene isn't silenced in FXTAS patients as it is in people with Fragile X; in fact, levels of the gene's product, FMRP, appear to be nearly normal. That casts suspicion on the mRNA that translates the gene's instructions into protein, says Paul Hagerman. In people with the premutation, *FMR1* mRNA bears an unusually high number of CGG repeats just as the gene itself does. Unexpectedly, however, people with the premutation make five to 10 times more *FMR1* mRNA than do those without it, Hagerman has found. "It's a puzzle," he says. "You'd expect it to go down, not up."

In a 2002 paper in *Brain*, the Hagermans and colleagues reported that the brains of four men who died with FXTAS were riddled with tiny blobs of protein and other material. These "inclusions" clustered inside the nuclei of neurons and astrocytes, a type of support cell, and contained high concentrations of *FMR1* mRNA. The team has now analyzed a total of 11 brains from FXTAS patients and found that those patients who had more CGG repeats in *FMR1* had more inclusions and died at a younger age than did men with fewer repeats. The findings appeared in the January issue of *Brain*.

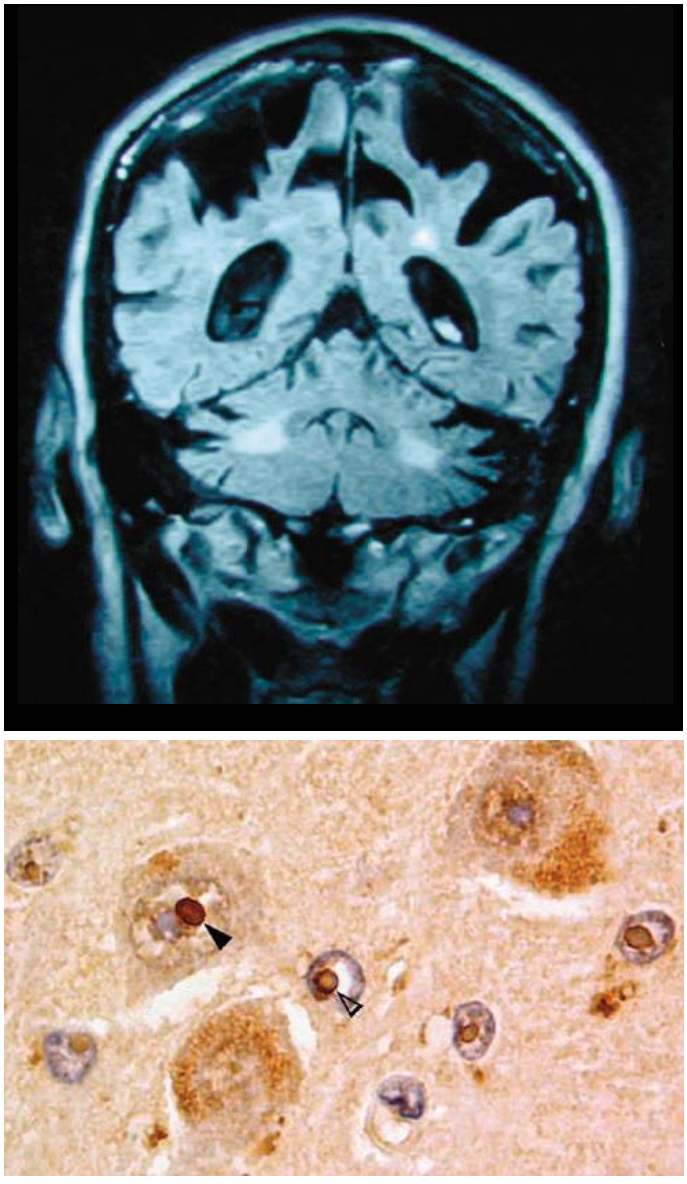
In a second study reported in the same issue, the Hagermans' team identified more than 20 proteins inside the inclusions. One, lamin A/C, is especially interesting, says Paul Hagerman. Lamin A/C is a filamentlike protein that among other duties supports the membrane forming the nucleus of a cell. Hagerman suspects that the CGG repeats make the *FMR1* mRNA an unusually attractive binding target for various proteins, including lamin A/C. According to this theory, the mRNA sops up the proteins, preventing them from doing their usual chores inside the cell.

Indeed, adding *FMR1* mRNA with extra CGG repeats to cultured human neural cells disrupts lamin, Hagerman and colleagues reported in the 1 December 2005 issue of *Human Molecular Genetics*. "Normally, you see a beautiful ring around the nuclear membrane when you stain for lamin," Hagerman says. "But when you express the repeats, the ring breaks down and just forms clumps." Hagerman says it's too early to say how lamin A/C disruptions might cause axon degeneration, but he notes that lamin irregularities have been implicated in another neurodegenerative disorder, Charcot-Marie-Tooth disease.

Other researchers agree that the sticky mRNA scenario is plausible. Many see a parallel with an inherited muscle disorder called myotonic dystrophy. In the most common form, the problem stems from a mutant gene whose mRNA bears abnormal repetition of the nucleotide sequence CTG. Various proteins glom onto the mRNA's repeat region and neglect their usual duties, causing the cells to malfunction. Although many inherited disorders are caused by a mutation that silences a gene (as in Fragile X) or results in a malformed, toxic protein (as in Huntington's disease), myotonic dystrophy is the only disorder known to be caused by abnormal RNA.

"The concept of RNA toxicity is really just emerging," says Emory geneticist Peng Jin. Like the Hagermans, Jin suspects that such toxicity is the root cause of FXTAS. In collaboration with Warren and others, he published a paper in *Neuron* in 2003 showing that expanded CGG repeats in *FMR1* mRNA causes neurodegeneration in fruit flies. The flies also had inclusions in brain cells similar to those seen in FXTAS patients.

At the same time, researchers have begun studying the effects of *FMR1* premutations in animals with nervous systems more closely resembling our own. Last year, Ben Oostra and colleagues at Erasmus University in Rotterdam, the Netherlands, described FXTAS-like symptoms in male mice with 98 CGG repeats in the gene. "If you look at the mice when they're young, there's no difference" between the mutants and their normal brethren, says Oostra. But by 1 year—middle age for a mouse—the mice with the premutation develop symptoms of ataxia, Oostra says. The Dutch researchers also reported in the 30 July 2005 issue of *Behavioral Brain Research* that these mice become unusually skittish and have memory deficits that grow worse with age—both features that have been described in people with FXTAS.



**Signs of trouble.** An MRI scan (*top*) reveals degeneration characteristic of FXTAS. In postmortem tissue (*bottom*), protein inclusions in neurons (dark arrow) and astrocytes (open arrow) are hallmarks of the disease.

CREDITS (TOP TO BOTTOM): P. J. HAGERMAN; COURTESY OF C. GRECO/UC DAVIS

### Missed diagnosis

Physicians are still clarifying the symptoms of FXTAS in people. Recent studies have found that memory and cognitive problems often follow the ataxia and tremor, says Randi Hagerman. Some patients act as if they have frontosubcortical dementia, she and colleagues reported in the January issue of the *Journal of Clinical Psychiatry*. This type of dementia is characterized by difficulty controlling mental processes, and patients often have trouble formulating plans, focusing their attention, or knowing what's appropriate

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## A Fix for Fragile X Syndrome?

The cognitive and behavioral problems associated with Fragile X syndrome would seem to be irreversible, because they're caused by a genetic glitch that derails the development of the nervous system. Yet much to their surprise, some researchers say that many of these problems might be fixable with drugs. Within a year, they predict, clinical trials will be under way to test compounds that target a family of receptors believed to play a critical role in symptoms of the inherited disorder. "I've been working on Fragile X for 25 years, and I never thought I'd be working on a drug," says Stephen Warren, a geneticist at Emory University in Atlanta, Georgia.

The drugs Warren and others envision would target the so-called metabotropic glutamate receptor (mGluR) that sits on the surface of neurons. The idea that mGluRs might be important actors in Fragile X arose from a chance meeting several years ago between Warren and Mark Bear, a neuroscientist now at the Massachusetts Institute of Technology in Cambridge.

At a gathering of Howard Hughes Medical Institute investigators, Bear had described recent work suggesting that mGluRs are crucial for weakening synaptic connections between neurons in the hippocampus, a brain region involved in learning and memory. Such weakening, called long-term depression (LTD), is an important form of neural plasticity during brain development and may underlie changes in neural connectivity that support learning later in life. Bear's lab had discovered that LTD requires activation of mGluRs in order to translate crucial mRNA molecules floating near synapses into proteins.

Warren, who'd been studying FMRP, the protein that's missing in Fragile X, happened to sit next to Bear after the mGluR talk and introduced himself. Warren's team had found that FMRP suppresses the kind of protein synthesis that Bear had discovered to be essential for LTD. "We began an animated conversation," Bear says. By the end of it, Warren had agreed to send Bear some Fragile X mice, which have a mutation that mimics that in people with the syndrome.

Warren, Bear, and colleagues reported in 2002 that these mice have enhanced LTD compared to normal mice. This propensity to weaken synapses could slow brain maturation and contribute to the developmental and cognitive problems seen in people with Fragile X, Bear and Warren later argued in an article published in 2004 in *Trends in Neuroscience*. Based on this and other evidence, the authors proposed that drugs that block mGluRs could mitigate many symptoms of Fragile X by performing the job normally done by FMRP: putting a check on mGluR-mediated protein synthesis.

> **"I've been working on Fragile X for 25 years,** and I never thought I'd be working on a drug."
>
> —Stephen Warren, Emory University

Tests with such compounds in fly and mouse models of Fragile X have lent support for that suggestion. Flies missing the gene that encodes FMRP have altered courtship behavior, impaired learning and memory, and altered anatomy in a brain structure involved in learning—all of which can be reversed with a compound that blocks mGluRs, a team led by Thomas Jongens at the University of Pennsylvania reported in the 3 March 2005 issue of *Neuron*.

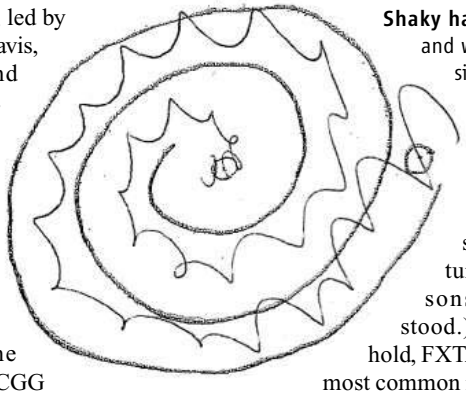
Blockers of mGluRs also reverse impairments in Fragile X mice, at least in some experiments, says Ben Oostra of Erasmus University in Rotterdam, the Netherlands. Oostra suspects, however, that mGluR blockers won't alleviate all Fragile X symptoms. "I am optimistic that some defects like epilepsy and autistic behavior and maybe hyperactivity might benefit, but I am more pessimistic about other parts of the phenotype of Fragile X," he says.

Bear and Warren have each started a company to investigate candidate drugs. Bear is testing mGluR blockers under license from Merck in animals, whereas Warren is screening compounds that may interfere with related cell signaling pathways. "We're doing animal toxicity studies now to ensure they're safe," says Bear. "So far they look very safe." He hopes to soon secure permission for a clinical trial.

**–G.M.**

behavior. "We had one guy [with FXTAS] whose family told us when they went out for dinner, he went to the bathroom and came back with the toilet seat on his head as a joke," Hagerman says.

A three-center study, led by Paul Hagerman at UC Davis, Leehey in Denver, and Elizabeth Berry-Kravis at Rush University Medical College in Chicago, Illinois, will help nail down the symptoms of the disorder and describe how it progresses. A major goal, says Berry-Kravis, is to determine whether the number of CGG repeats predicts the severity and type of symptoms.



**Shaky hands.** Difficulty drawing and writing is one of the first signs of FXTAS.

CREDIT: ELIZABETH BERRY-KRAVIS AND ALLISON SUMIS/RUSH UNIVERSITY MEDICAL CENTER

For men with the premutation, the prevalence of FXTAS increases sharply with age, from 17% of those in their 50s to 38% of those in their 60s to 75% of those 80 or older, the Hagermans and others reported in the *Journal of the American Medical Association* (*JAMA*) in 2004. The researchers estimated that the disorder will strike one in 3000 men in the general population. (FXTAS appears to be very rare among women, although women with the premutation are susceptible to premature menopause for reasons that aren't understood.) If these calculations hold, FXTAS would be one of the most common neurodegenerative disorders linked to a specific gene, says Berry-Kravis, one of the authors of the *JAMA* study. Huntington's disease, which has been considered relatively common for this type of disorder, only strikes about one in 10,000 people, for example. Other disorders that have a genetic component but aren't tied to a single gene are far more common. Parkinson's disease falls into this category and affects about one in 100 people.

Misdiagnosing FXTAS as Parkinson's disease or another illness can lead to treatments that are futile or worse, notes Paul Hagerman. "I know of four cases where people had neurosurgery to implant shunts," he says. The patients were diagnosed with hydrocephaly because their brains had atrophied, making the fluid-filled ventricles deep in the brain look abnormally large.

The other reason patients need to know if they have FXTAS is the implications for genetic counseling, says Randi Hagerman. As awareness of FXTAS has grown, neurologists have begun to identify the disorder in men whose families include no one with Fragile X syndrome, she says. Some of these men have daughters who may be thinking about starting families, Hagerman notes, and the pattern of inheritance means that all these women carry the premutation: "They didn't know they were carriers, and that's very important information for them."

**–GREG MILLER**

PROFILE: ALICE DAUTRY

# After the Storm, New Pasteur Chief Treads Softly

**Following a year of chaos and revolt, the new Pasteur Institute president aims to steady nerves before she continues the path of reforms**

**PARIS**—After 6 months in the job, Alice Dautry has already proved her mettle in one respect: The Pasteur Institute is working again. The lab had been paralyzed with dissension when Dautry, 55, was appointed president in September 2005. Over a tumultuous year, the staff had revolted against a controversial plan to move several research units to a suburb. Many also objected to what they called the aggressive management style of Dautry's predecessor Philippe Kourilsky. The resulting fights had poisoned the atmosphere and stifled research. In January 2005, the entire board of directors stepped down in an attempt to solve the crisis.

**Guarding the heritage.** Alice Dautry, who took the helm of the Pasteur Institute in September, wants to strengthen its efforts in virology.

Five months later, after a new board had been elected, Kourilsky was forced to leave.

Pasteur scientists say Dautry's personal style—she comes across as likable, informal, and modest—has helped heal wounds and restore a sense of normality. But now that the honeymoon is over, Dautry is facing the same challenges as her predecessor: keeping Pasteur at the top of the global research league, promoting excellence despite a fragile budget, recruiting talent at modest salaries to facilities that are now quite aged, and countering the general malaise that afflicts French science. And her solutions may turn out to be not radically different from what Kourilsky had in mind. For example, Dautry doesn't even rule out the possibility of a new relocation plan. But her style is unquestionably more diplomatic.

Dautry has a doctorate in physics from Paris-Sud University and a master's in molecular biology from Stony Brook University in New York. She was lured to Pasteur by the famed biologist, Nobel laureate, and World War II resistance fighter Jacques Monod in 1975. "I was very young and very, very impressed," she says. She started her own group in 1984 after a 2-year stint at the Massachusetts Institute of Technology and has headed a 12-person unit called Biology of Cell Interactions since 1992. She is the first woman at Pasteur's helm, although she says the director's gender is irrelevant.

Despite the recent mayhem, the lab Dautry inherits is in better shape now than it was 5 years ago, many Pasteur scientists agree. Kourilsky tried to make the institute more attractive to young scientists by offering them small research groups for 5 years, strengthened evaluations based on merit, sought to create new international collaborations, and tried to increase revenues from patents to boost a straining budget. Whatever his management flaws, continuing Kourilsky's policies is "extremely important," an external panel wrote in a May 2005 letter to the newly elected president of the board of directors, François Ailleret.

Dautry declined to comment directly on the letter, but in an interview with *Science*, she emphasized many of the themes Kourilsky promoted. She says she wants to lure more young scientists and give them a stronger voice in the institute; like Kourilsky, she wants to expand collaborations abroad; and a certain resentment against commerce at Pasteur notwithstanding, she also wants to coach Pasteur staff on the importance of patents. But so far, she has moved cautiously. When she introduced herself to French journalists during a press conference in February, she announced some shifts in emphasis but no grand plans.

## Close community

In contrast to Kourilsky, who envisioned continued rapid growth, Dautry isn't convinced that Pasteur's staff needs to expand much further. With some 1400 scientists and 1200 support staff, the lab is still a close community. People know each other, and this stimulates collaboration and creativity, she says: "If we do grow much more, we become something else, and we lose some of that synergy."

Boosting Pasteur's efforts in virology is necessary, says Dautry, because so many emerging threats—including SARS and avian influenza—are caused by viruses. But any new investments won't be to the detriment of traditional Pasteur strongholds such as parasitology and bacteriology, Dautry says. Nor does she plan to quit topics such as neurobiology and developmental biology, both of which are considered expendable by some in the event of a complete focus on infectious diseases.

Instead, she says she can strengthen virus research by seeking more outside collaborations and by breaking down the walls within the institute itself. In a recent reorganization, for instance, all virologists were united in a new department, and Pasteur quickly launched a broad research program in response to the outbreak of Chikungunya, a relatively unknown mosquito-borne virus that sickened hundreds of thousands on the French island of La Réunion in the Indian Ocean (*Science*, 24 February, p. 1085).

Dautry also has high hopes for collaboration with the 28 Pasteur Institutes around the world, most of them in developing countries. Essentially a holdover from the colonial era, the majority are now independent, and their number is still growing. (The latest, nestled in a former French missionary building in Shanghai, is a 2-year-old collaboration with the Chinese Academy of Sciences. Dautry says another deal may soon be signed.) The mother ship in Paris helps the labs build research capacity and in return gets to study diseases where they happen. And in an age of one emerging disease after another, "it's really

CREDIT: STEPHANE DE SAKUTIN/AFP/GETTY IMAGES

a unique opportunity," says cell biologist and Nobelist Paul Nurse of Rockefeller University in New York City, a member of the external panel who was asked earlier this month by Dautry to help set up a new one.

The workspace in Paris, meanwhile, remains a sensitive issue. Kourilsky was ultimately undone by his plan to move part of the lab from its beloved Paris campus—where Louis Pasteur is buried, and his home and lab turned into a museum—to a building donated by drug company Pfizer on an industrial site in the southern suburb of Fresnes. The plan, which Kourilsky deemed essential for growth, was dropped when mediator John Skehel, director of Britain's National Institute for Medical Research in London, concluded the move was unnecessary (*Science*, 25 February 2005, p. 1183).

Although that ruled out Fresnes, Dautry says she hasn't closed the book on all new locations, provided they can add something that's valuable for research, such as increased collaboration with nearby hospitals, research centers, or technology hubs. The problem with Fresnes, she says, was that "it was just a site to move people to. It wasn't part of a clear project to develop the institute." The discussion will start anew, "very calmly," in 6 or 12 months, she says. Meanwhile, Dautry faces challenges on the existing campus: A complex refurbishment requires temporarily shuffling groups between already cramped buildings, and neighbors in Paris's 15th arrondissement have voiced opposition—and could delay—construction of a planned new building.

So far, Pasteur staff members have given her the benefit of the doubt. "We're in a period of mutual observation. … It's wait and see," says Elise Caliot, a member of the Workforce Delegates—one of two bodies representing workers at the institute—on behalf of the trade union CGT. A first test of the new relationship will come soon, when collective salary negotiations begin, says Caliot. (The unions are aiming for a raise.) But she approves of the fact that Dautry has opted for a small, three-member management team from within Pasteur that is in frequent dialogue with the work floor. Caliot says that Kourilsky recruited managers from the world of business who didn't fit in with Pasteur's academic culture; Dautry hasn't made that error, she adds.

Antoine Danchin, who heads the Genomes and Genetics department at Pasteur, says that despite her affable manner, Dautry "may be more authoritarian than one would think. … She knows what she wants to do." The biggest problem facing her, however, is luring fresh talent at a time when lab budgets are meager and researchers in their late 30s make hardly any more money than Paris Metro drivers. "Her main problem is not going to be within Pasteur," says Danchin. "It's going to be the crisis in France."

**–MARTIN ENSERINK**

**Soured.** A foreign bacterium deforms citrus such as this pomelo and gives it an acrid taste.

CREDIT: XIAOAN SUN AND SUSAN HALBERT

AGRICULTURE

# New Disease Endangers Florida's Already-Suffering Citrus Trees

**Researchers are mobilizing to stop a wily bacterial marauder spread by invasive insects, but massive losses appear inevitable**

Things are going from bad to worse for citrus growers in Florida, the state that produces most of the oranges, grapefruit, and limes grown in the United States. The $9 billion industry has spent much of the past decade wrestling with an epidemic of canker that cut production by roughly a third. In a long, drawn-out effort to eradicate the wind-borne bacteria, 12.7 million trees—among them 10% of Florida's commercial acreage—have been cut down at a cost of $600 million including compensation. But in January, the U.S. Department of Agriculture (USDA) decided that eradicating canker was a lost cause and halted its efforts.

Even more dire news arrived last August, when a team of scientists discovered two cases of citrus greening in South Florida. Greening is "probably the worst citrus disease in the world," says Harold Browning, who directs the University of Florida's Citrus Research and Education Center (CREC) in Lake Alfred. Whereas canker just makes trees less productive and blemishes fruit, greening renders it totally unusable and eventually kills the trees.

Researchers think it will be impossible to stop the disease from conquering the state, because the pathogen, a bacterium called *Candidatus liberibacter*, is spread by an introduced insect that has run rampant across Florida. Even just getting a firm grasp of the situation is devilishly tricky: Infected and contagious trees don't show symptoms for several years. "It's commonly widespread before it's discovered," says plant epidemiologist Timothy Gottwald of USDA in Fort Pierce, Florida. "It's almost below the threshold of detection until it explodes."

The goal now is to contain that explosion as much as possible. A nationwide survey of citrus states is under way, and researchers are racing to sequence the genome of the bacterium in hope of developing new tests to detect the microbe. At the same time, research is also aimed at finding ways to help growers manage the disease, such as the most effective way to spray insecticides. A preliminary response plan released last month by USDA and the Florida Department of Agriculture and Consumer Services (FDACS) lays out strategies for keeping nurseries disease-free. But with two major pathogens likely entrenched in Florida—and more looming on the horizon—some researchers say only the distant prospect of citrus trees engineered for resistance may save the industry.

### Worldwide menace

There's good reason to be concerned about greening. The disease has devastated citrus crops across the world, ravaged by three species of the bacterium. Trees become stunted and lose leaves. Instead of living for decades, they die after just 5 to 8 years. Their fruit can be lopsided, small, and green (hence the name), and they make vile juice. "It tastes like jet fuel mixed with Vicks VapoRub," says David Hall, an entomologist with USDA in Fort Pierce, Florida. Throughout China and India, the Asian species of *C. liberibacter*—now present in

Florida—has made production economically impossible. The vector is an aphidlike insect called a psyllid. If psyllids are abundant, greening can wipe out a grove in just a few years.

Florida entomologists first spotted the Asian citrus psyllid (*Diaphorina citri*) in 1998. It likely arrived on infested plant material and since then has hitchhiked across the state on orange jasmine (*Murraya paniculata*), an ornamental plant shipped in large quantities by discount stores. Two years ago, USDA and FDACS began conducting limited scouting trips for greening.

In August, inspectors spotted a pomelo tree that had lost its leaves and had misshapen fruit on a small farm near Florida City, in the southern end of the state. "The alarm bells really started ringing," Gottwald recalls. After two government labs confirmed the presence of greening, more survey teams were sent out and quickly found the disease as far as 270 kilometers north. "It became clear that this had spread beyond anything we could get our arms around," Gottwald says. Last month, greening was confirmed in DeSoto County, the 12th county to date. A more detailed statewide survey of incidence and severity will begin next month to look for hot spots. And the USDA has begun coordinating a broader 6-month-long survey of citrus-producing states—California, Arizona, Texas, and Louisiana—looking for both greening and psyllids.

Accurate surveying is not easy. Detecting early stages of greening—before the fruit is affected—is a major challenge, as the symptoms resemble nutrient deficiency. "It's easy to get fooled," says plant pathologist Ronald Brlansky of the University of Florida's CREC in Lake Alfred. The current molecular test, based on the polymerase chain reaction (PCR), is reliable for confirming a symptomatic infection, but only when levels of bacterium in the sample are high. It also looks for just two genetic loci, which means the test could be missing new species of the pathogen, says plant pathologist and geneticist Dean Gabriel of the University of Florida (UF), Gainesville. Indeed, several highly symptomatic trees have tested negative for the Asian species by PCR, Gabriel says.

Soon after greening was discovered, USDA gave Gabriel a $117,000 grant to sequence the *C. liberibacter* genome. Because researchers don't know how to grow the organism in the lab, what would otherwise be a trivial sequencing effort is painstaking work, so Gabriel's group has to rely on infected trees in a biosafety level 3 facility. Gabriel expects to complete the sequencing in the next 6 months.

The inability to culture the bacterium is a serious problem. Unless researchers can grow the bacterium in petri dishes, they are handicapped in discovering which genes make it so destructive. They also can't screen for antimicrobial compounds in vitro or generate various detection methods such as those that rely on monoclonal antibodies. But culturing the bug is a tall order: "Some of the best labs in the world haven't been able to achieve this," says Gottwald, who thinks it could require concerted effort by multiple labs and millions of dollars. Brlansky has begun work in this direction with Michael Davis of UF Homestead and plant pathologist John Hartung of USDA in Beltsville, Maryland.

**In quarantine.** Chinese box oranges are among the plants being inspected for the Asian citrus psyllid (*inset*) in Florida nurseries, which have suffered steep losses of citrus.

In the meantime, officials are trying to figure out the best way to manage the disease. Last month, USDA and FDACS released a draft response plan for greening that specifies measures for slowing the spread and preventing it from reaching other states. The major steps include inspecting nursery stock to make sure it's disease-free. Nurseries will be required to grow their citrus in screened greenhouses in approved locations. But exactly how far away nurseries must be isolated from groves is controversial; moving facilities could be an enormous expense for nurseries that are already suffering from major losses due to canker. The regulation has not yet been finalized.

The answer depends on unknowns such as how far psyllids can fly. USDA's Hall and other researchers are studying the psyllid, trying to learn more about its ecology. In addition to helping set guidance for nurseries, the information could help determine the most effective times and doses for citrus growers to spray insecticides. Experts say biocontrol is unlikely to work well in Florida, in part because parasites hold back the wasps used for biocontrol. "With greening, there really aren't many tools," says UF's Browning.

One major question is how big a problem the infected trees of homeowners will be. Although these trees will be sources of bacterium until they die, the state won't require their removal, says Timothy Schubert, a plant pathologist with FDACS, citing "political realities." His department will try to educate homeowners about the need to quickly destroy sick trees, he says, but such attempts were only marginally effective with canker.

Another question is whether orange jasmine is a host for greening. If so, there would be concerns that shipments of the plant might have spread greening to other states that already have psyllids, such as Texas. (After the discovery of greening, Florida quarantined citrus and citrus relatives in nurseries.) In Brazil, scientists are convinced that orange jasmine can be infected by their species of the bacterium. Some jasmine tested in the United States contains faint, ambiguous signs of DNA from the Asian species of bacterium that's present in Florida. Researchers at USDA's labs in Fort Detrick, Maryland, are continuing to investigate.

Even though only 615 trees have been confirmed positive so far, Schubert and others expect the number to rise drastically, with a major impact on the citrus industry. The problem will be compounded by a shortage of healthy trees to replace them, due to the crisis at nurseries. In the long term, says Schubert, "the brightest hope of any semblance of an industry like we have had up till now is genetic engineering." Gabriel and a few other researchers are working on engineering resistance to canker and other diseases into citrus, but no one has yet figured out how to develop resistance to greening. "That's going to take years," says Gabriel.

**–ERIK STOKSTAD**

CREDITS: FLORIDA DEPARTMENT OF AGRICULTURE AND CONSUMER SERVICES DIVISION OF PLANT INDUSTRY

EDITED BY YUDHIJIT BHATTACHARJEE

## Pioneers

**TO HEAL A NATION.** As a medical student in the Democratic Republic of Congo in the 1970s, Oscar Kashala led a student movement against corruption and human rights violations in the country. Decades later, as an oncology researcher working in the American pharmaceutical industry, Kashala encouraged his colleagues to develop vaccines for African strains of malaria and other diseases. Now he's hoping to make a bigger difference by winning Congo's presidency.

Kashala has taken leave from his job at Millennium Pharmaceuticals Inc. in Boston, Massachusetts, to run against incumbent Joseph Kabila in elections scheduled for June. Although observers think his chances are slim, he has hit the campaign trail with gusto. Public health is part of his platform—"many hospitals [in the country] don't have mattresses," he says. He says his expertise and international ties would help in that effort, going hand-in-hand with economic and government reforms aimed at bringing development and peace to the strife-torn nation.

AIDS researcher Max Essex, who was Kashala's adviser at Harvard, says Kashala is one of many talented foreign-born scientists he's trained who contribute to their countries. "But none of them try to go back as a political leader," he says, adding that Kashala's qualities of being "in charge" and "well liked" bode well for his candidacy in the country's first election since 1970.

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CREDITS (TOP TO BOTTOM): OSCAR KASHALA; CALTECH; JOYCE RAVID

## AWARDS

**A PRECIOUS RESOURCE.** A Canadian ecologist and a Russian hydrologist share the $200,000 Tyler Prize for Environmental Achievement for efforts to protect the world's water resources.

David Schindler of the University of Alberta, Canada, has done pioneering experiments showing how acid rain destroys freshwater lakes and how phosphorus promotes uncontrolled growth of algae. His research persuaded politicians around the world to ban the use of phosphorus in detergents.

Igor Shiklomanov, who directs Russia's State Hydrological Institute in St. Petersburg, has studied how local water consumption for domestic and agricultural purposes affects global water supplies.

**ENERGY PRIZE.** Three pioneers of nuclear fusion will share the $1.1 million Global Energy International Prize. Evgeniy Velikhov, director of the Kurchatov Nuclear Research Center in Moscow; Yoshikawa Masaji, former president of the Japan Atomic Energy Research Institute; and Robert Aymar, director-general of the CERN particle physics lab in Geneva, Switzerland, are being honored for laying the scientific and engineering foundations of the planned International Thermonuclear Experimental Reactor. The annual prize is funded by three Russian power companies.

**UNTANGLING WAVES.** A French-born mathematician whose work could improve technologies for applications including medical imaging and DNA analysis has won the National Science Foundation's (NSF's) top annual prize for researchers aged 35 or younger. Emmanuel Candes, a professor at the California Institute of Technology in Pasadena, will receive a 3-year, $500,000 grant as recipient of the Waterman Award.

Candes, 35, works on harmonic analysis, a field devoted to separating out single waves from complex signals for analysis and processing. His research "promises to take the field to a whole new level," says John Cozzens, an NSF program officer in the computing directorate.

## MOVERS

**MASSIVE UNDERTAKING.** German physicist Norbert Holtkamp, who is currently working on the Spallation Neutron Source at Oak Ridge National Laboratory in Tennessee, has been chosen to lead the building of the International Thermonuclear Experimental Reactor. Named the principal deputy director-general of the seven-nation project, Holtkamp will serve as the key technical supervisor for the construction of the reactor in Cadarache, France, over the next decade.

## CAMPAIGNS

**ORGAN OF CHANGE.** In August 2004, psychiatrist Sally Satel learned from routine lab tests that her kidneys were failing. Her options were limited to putting her name on a waiting list for cadaver organs, behind some 88,000 Americans, and registering on a Web site that matches willing live donors with recipients who pay to be listed. Those avenues didn't work out, but Satel was fortunate to have a friend offer a kidney to her. The transplant was performed last month, and both women are recovering well.

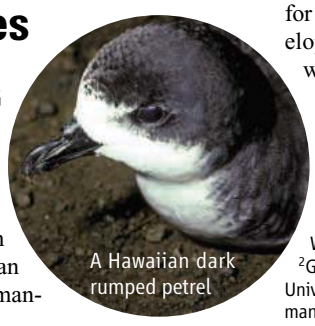
Satel, a health policy researcher at the American Enterprise Institute (AEI) in Washington, D.C., says the experience awakened her to the "horribly broken" state of organ transplantation rules in the United States. To improve matters, she is organizing a conference at AEI in June to discuss how the system could be made more efficient. Satel advocates that individuals be compensated for donating their organs, a practice currently prohibited by the 1984 Organ Transplantation Act.

To prevent the exploitation of poor people under such a free-market regime, she says, the government could institute safeguards such as giving donors an income tax holiday for a year—which would appeal more to wealthy potential donors than to poor ones. Also, Satel says, the government could enact "presumed consent," according to which individuals would automatically be considered organ donors after death. "To ask people to wait in line is almost cruel when there are other options," she says.

# LETTERS

*edited by Etta Kavanagh*

## Ongoing Threats to Endemic Species



A Hawaiian dark rumped petrel

CAROLYN GRAMLING'S ARTICLE "HAWAII'S CORAL TREES FEEL THE STING of foreign wasps" (News Focus, 16 Dec. 2005, p. 1759) highlights the risk posed by a nonnative wasp. This is only the most recent example of the vulnerability of endemic Hawaiian species to foreign invaders (*1*). For example, the continued existence of the Hawaiian dark rumped petrel (*Pterodroma phaeopygia sandwichensis*) depends on control of nonnative predators at its breeding colonies. Such threats can at best be managed rather than eliminated. Continuing conservation management thus is necessary to maintain such species.

The need for ongoing management to maintain species is one characteristic of what has recently been defined as "conservation-reliant species" (*2*). Such species face threats that are pervasive, recurring, and cannot be eliminated (e.g., nest parasites, predators, and dependence on disturbance habitat regimes). Without species-specific management, conservation-reliant species are at risk of extinction.

The need for ongoing management runs counter to traditional assumptions. In the Endangered Species Act, for example, Congress assumed that, after a species is listed, the threats to its existence will be identified and eliminated so that the species can be taken off the list as recovered. This assumption is proving to be false (*2*).

Given projections of increased globalization with its resulting homogenization and the concomitant fragmentation of native habitats, the threats of nonnative species can only be expected to increase. Thus, the threat posed to the survival of the coral tree by nonnative wasps, rather than being an interesting observation from an isolated island ecosystem, offers a window to the future challenges facing conservation biology in its efforts to conserve imperiled species worldwide.

J. MICHAEL SCOTT[1] AND DALE D. GOBLE[2]

[1]U.S. Geological Survey, University of Idaho, Moscow, ID 83844–1141, USA. [2]College of Law, University of Idaho, Moscow, ID 83844–2321, USA.

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## A Scientific Supercourse

IN THEIR RECENT EDITORIAL "DOING MORE FOR Kate"(16 Dec., 2005, p. 1741), T. Cech and D. Kennedy describe the need to revitalize science education. They wrote, "[We need to] teach better with less struggle." In an effort to empower higher education teachers, we built a Global Health Network Supercourse library of 2500 PowerPoint lectures on public health and prevention (*1*). The Supercourse, funded by the National Institutes of Health, has a network of more than 32,000 volunteer scientists from 151 countries.

The realization of the Supercourse is evidence that scientists in one field can network together to share their best lectures. Judging by the many thousands of teachers and students we are drawing to our Web site, we have been highly successful in improving training, research, and collaboration in the field of public health and prevention.

The first step to expanding the system to all of science is to build a collection of the best lectures from the top scientists, such as Nobel Prize winners and members of the U.S. Institute of Medicine and National Academy of Sciences of the United States. The ultimate expansion will be to all scientists worldwide, with a faculty of a million and 300,000 lectures available to all. If we could achieve this goal, we would reduce the time it takes for scientific information to reach the classroom from 7 years to 7 minutes. At the same time, we could improve science training for millions, which is especially needed in developing countries. A Supercourse of Science would provide a new, more efficient means of teaching.

RONALD E. LAPORTE,[1,2]* GILBERT S. OMENN,[3] ISMAIL SERAGELDIN,[4] VINTON G. CERF,[5] FAINA LINKOV[2]

[1]Director, Disease Monitoring and Telecommunications, WHO Collaborating Center, Department of Epidemiology, [2]Graduate School of Public Health, 3512 Fifth Avenue, University of Pittsburgh, Pittsburgh, PA 15261, USA. [3]Chairman of AAAS Board of Directors, and Department of Internal Medicine, Human Genetics and Public Health, University of Michigan Medical School, A510 MSRB I, Ann Arbor, MI 48109–0656, USA. [4]Director, Library of Alexandria, Shatby 21256, Alexandria, Egypt. [5]Chief Internet Evangelist, Google/Regus, 13800 Coppermine Road, Suite 384, Herndon, VA 20171, USA.

*To whom correspondence should be addressed. E-mail: rlaporte@pitt.edu

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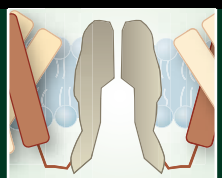
## Marine Parks Need Sharks?

IN THEIR REPORT "FISHING, TROPHIC CASCADES, and the process of grazing on coral reefs" (6 Jan., p. 98), P. J. Mumby *et al.* showed that increased grouper biomass within the Exuma Cays Land and Sea Park (ECLSP) did not acutely impair the grazing capacity of parrotfish populations (a major prey item), alleviating concerns that marine reserves may cause trophic cascades that will impede the recovery of imperiled Caribbean coral reefs. Although Mumby *et al.* suggest that this finding was primarily due to coincident beneficial reserve effects for parrotfish, we suggest that the abundance of sharks in the ECLSP, due to limited shark fisheries in the Bahamas (*1*), may have been an important contributing factor.

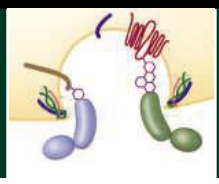
Modeling suggests that sharks are important regulators of grouper biomass on Caribbean reefs (*2*). Thus, establishment of reserves in areas where sharks are severely overfished will release grouper from two key predators (humans and sharks), likely driving a more explosive increase in their biomass than observed in the ECLSP

CREDIT: R. SHALLENBERGER/VIREO



Voltage-dependent proton channels

534



Botox receptor

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where sharks are common. This in turn may drive an acute reduction in parrotfish biomass and grazing capacity (*2*).

Because many shark populations in the Atlantic are severely overfished and are unlikely to respond to protection as quickly as teleosts (*3*), there is an urgent need for comparative studies of trophic cascades and grazing in recently established coral reef reserves with and without healthy populations of sharks. Moreover, ecosystem-based fisheries management (*4*) and novel reserve designs [e.g., large, zoned reserves (*5*)] aimed at maintaining multiple ecosystem components, including these top predators (*2*, *5*), are probably needed on Caribbean coral reefs to maintain parrotfish grazing capacity and other critical ecosystem processes.

A tiger shark in the Bahamas.

CREDIT: CORBIS

DEMIAN D. F. CHAPMAN,[1] ELLEN K. PIKITCH,[2] ELIZABETH A. BABCOCK[3]

[1]Guy Harvey Research Institute, Nova Southeastern University, Dania Beach, FL 33004, USA. [2]Pew Institute for Ocean Science, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 126 East 56th Street, New York, NY 10022, USA. [3]Pew Institute for Ocean Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA.

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1. According to the Food and Agriculture Organization, reported shark landings in the Bahamas were <1 mt in 1999, which in part reflects a nationwide longline fishing ban.
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## Response

WE DISAGREE WITH CHAPMAN *ET AL.*'S ASSERTION that potentially high levels of shark abundance were an important contributor to the observed increase in parrotfish grazing within the Exuma Cays Land and Sea Park (ECLSP).

First, a key finding of our study was that larger-bodied parrotfish species achieve an escape from grouper predation and therefore the interaction between groupers and parrotfish is surprisingly weak, reducing parrotfish grazing by only 4 to 8%. Although we cannot discount the possibility that even greater biomasses of grouper could eventually constrain grazing, current biomasses are already at least double that of other reported levels in the Caribbean.

Second, there is no empirical evidence that sharks regulate grouper biomass. In Bascompte *et al.* (*1*), the modeling of shark-grouper interactions ignores active prey selection and prey size refugia and may prove to be simplistic once studied empirically. Thus, we agree that the ecosystem functioning of sharks warrants further study.

Third, it is difficult to imagine that sharks would have a strong impact on groupers but not on parrotfishes. Indeed, Bascompte *et al.* (*1*) found that only four of 200 tritrophic relationships containing sharks, groupers, and parrotfish contained two or three strong inter-

actions. Of those four, two included direct negative impacts of sharks on parrotfish that would tend to counteract the positive indirect impact postulated by Chapman *et al*. Moreover, none of these tritrophic food chains involved the large-bodied species of parrotfishes, which undertake most of the grazing.

Even if sharks are unusually abundant in the Bahamas, their direct impacts are unlikely to differ across reserve boundaries because the shark species that most likely feed on groupers (and large parrotfish) range over large areas (*2*). Therefore, our results show that despite background shark predation, both groupers and parrotfish can achieve high biomasses when fishing levels are vastly reduced.

The key process driving these patterns is fishing mortality. The impact of a reserve depends on both the complexities of trophic cascades within the reserve and the differential in fishing mortality across its boundaries [our Report, (*3*)]. Our paper and that of Bascompte *et al.* (*1*) show that trophic cascades can be highly complex and occasionally surprisingly weak. We also show that even minor reductions in fishing mortality can overwhelm the negative influences of weak trophic cascades. Therefore, reductions in fishing mortality within Caribbean reserves will almost always lead to a net increase in parrotfish grazing.

PETER J. MUMBY,[1] FIORENZA MICHELI,[2] CRAIG P. DAHLGREN,[3] STEVEN Y. LITVIN,[2] ANDREW B. GILL,[4] DANIEL R. BRUMBAUGH,[5,6] KENNETH BROAD,[7] JAMES N. SANCHIRICO,[8] CARRIE V. KAPPEL,[2] ALASTAIR R. HARBORNE,[1] KATHERINE E. HOLMES[5]

[1]Marine Spatial Ecology Lab, School of BioSciences, University of Exeter, Prince of Wales Road, Exeter EX4 4PS, UK. [2]Hopkins Marine Station, Stanford University, Oceanview Boulevard, Pacific Grove, CA 93950–3094, USA. [3]Perry Institute for Marine Science, 100 North U.S. Highway 1, Suite 202, Jupiter, FL 33477, USA. [4]Institute of Water and Environment, Cranfield University, Silsoe, Bedfordshire MK45 4DT, UK. [5]Center for Biodiversity and Conservation, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024–5192, USA. [6]Marine Protected Areas Science Institute, National MPA Center, 110 Shaffer Road, Santa Cruz, CA 95060, USA. [7]Rosenstiel School of Marine and Atmospheric Science, Division of Marine Affairs and Policy, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA. [8]Resources for the Future, 1616 P Street, NW, Washington, DC 20036, USA.

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## Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

## A Not-So-Abrupt Departure

GIVEN R. A. KERR'S PAST RECORD OF OUTSTANDING science journalism, we were surprised by the unfortunate errors and omissions in his ScienceScope piece "Knock hockey," surrounding issues of how scientists gauge temperature over the last two millennia (10 Mar., p. 1359). The assertion that "[Michael] Mann made himself scarce throughout the proceedings, even abruptly departing as [Stephen] McIntyre stood to make a final comment" is incorrect. Mann's early departure had to do with his teaching obligations and a limited flight schedule. Mann was unaware of who may have been making comments as he departed following the official close of the presentation period, and he has been responsive to all requests made of him by the committee.

Kerr incorrectly asserts that Mann and colleagues have "sworn off" the method used in their original work. As "sworn off" has the connotation of renouncing or conceding a flaw in past work, this is simply incorrect. Mann and colleagues have developed more sophisticated approaches, as should be expected over the decade that has passed since the original work was begun. The newer approaches yield an essentially indistinguishable result (*1*) but are immune to potential criticisms of the simpler, original methodology ("Estimates, uncertainties, and noise," Editors' Choice, 25 Nov. 2005, p. 1249). Kerr does make the key point that additional recent research provides "independent support for temperature trends resembling Mann's." He might also have noted that high-profile criticisms of Mann and colleagues' original work have now been shown to have been based on an incorrect implementation of their procedures (*2*).

MICHAEL E. MANN[1] AND MALCOLM K. HUGHES[2]

[1]Department of Meteorology and Earth and Environmental Systems Institute, Walker Building 503, Pennsylvania State University, University Park, PA 16802–5013, USA. [2]Laboratory of Tree Ring Research, University of Arizona, 105 West Stadium, Tucson, AZ 85721, USA.

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2. E. R. Wahl, D. M. Ritson, C. M. Ammann, *Science*, www.sciencemag.org/cgi/content/full/312/5773/529b.

## Mechanisms for Resistance in Soil

"SAMPLING THE ANTIBIOTIC RESISTOME" BY V. M. D'Costa *et al*. (Reports, 20 Jan., p. 374) presents evidence that multiple diverse mechanisms for resistance, particularly those that degrade antibacterials, are associated with microbes in different soil samples. The investigators assembled a group of 480 strains of *Streptomyces* and examined them for resistance to 21 different antibiotics, including all major types and targets of activity. The results were compelling; they found multiple mechanisms of resistance to both naturally occurring and synthetic antibiotics. Notably, a number of resistances were linked to enzymatic inactivation. The results of this Report indicate that there are mechanisms in soil for potentially resisting both current antibiotics and antibiotics that have yet to be developed. Analyzing soil can help determine the kinds of resistances that exist and the kinds of resistances that could eventually emerge clinically. The inactivation mechanisms for some of the older antibiotics have not yet become a clinical problem. Perhaps this type of analysis can shed light on whether they will in the future.

The second message we glean from this study is that new naturally occurring antimicrobials may be overlooked because they have been inactivated, either by the producer or by other organisms. Thus, we must work to develop new methods for finding antibiotics in soils in order to protect the antibiotics from degradation. The findings of D'Costa *et al*. are of particular interest to the Reservoirs of Antibiotic Resistance project of Alliance for the Prudent Use of Antibiotics (*1*), which focuses on reservoirs of antibiotic resistance genes that have the potential to affect the clinical efficacy of antimicrobials. Soil bacteria are an important source of new antibiotics; this study clearly suggests that soils offer a predictive look at drug resistance as well.

STUART B. LEVY

Center for Adaptation Genetics and Drug Resistance, Department of Molecular Biology and Microbiology and Department of Medicine, Tufts University School of Medicine, Boston, MA 02111, USA; President, Alliance for the Prudent Use of Antibiotics, 75 Kneeland Street, Boston, MA 02111–1901, USA.

**Reference**

1. Alliance for the Prudent Use of Antibiotics (www.apua.org).

IN THEIR REPORT "SAMPLING THE ANTIBIOTIC resistome" (20 Jan., p. 374), V. M. D'Costa and co-workers show that a subset of the soil microbial flora belonging to the genus *Streptomyces* provides a reservoir of resistance determinants (the antibiotic resistome) that have the potential to be mobilized into the microbial community. The concept of a nonmedical environmental gene pool has been recognized (*1*, *2*). Similar studies in other environments have yielded similar results, and we caution against the perception that microbial gene transfer occurs within strict environmental compartments. Twenty years ago, resistance profiles of 2000 fecal and nonfecal bacterial isolates from lakes of differing eutrophic status were assessed (*3*, *4*). It was shown that although a large lake directly received sewage effluent, the incidence of antibiotic resistance was higher in the bacteria isolated from the lake water than in those from the effluent. Furthermore, the incidence of antibiotic resistance in aquatic bacteria isolated from the lake was lower than in those isolated from two remote upland tarns. Although they were not totally isolated from man and other animals, the tarns did not receive sewage or other effluents and therefore the results were surprising. A possible explanation might be that increased resistance is associated with adaptation to nutrient-poor environments (*3*, *4*). This seemingly passive resistance should be taken into account in the context of the D'Costa *et al*. study, as it would elevate the significance of the resistome. Furthermore, a recent study by Riesenfeld *et al.* (*5*), cited by D'Costa *et al*., concluded that "soil bacteria are a reservoir of antibiotic resistance genes with greater genetic diversity than previously accounted for."

It is apparent that the soil environment resistome [D'Costa *et al*. and (*5*)] is a component of a global resistome. Bacteria do not abide by human-imposed boundaries, and in considering environmental antibiotic resistance, terrestrial and aquatic environments should not be perceived as separate compartments of the environment. Interaction at a microbial level is not confined to these boundaries (*6*, *7*). We reiterate the view of D'Costa *et al*. and the accompanying Perspective by A. Tomasz (20 Jan., p. 342): If the extent of the resistome implied by local studies [D'Costa *et al.* and (*3*, *5*)] is translated to a global scale, then we vastly underestimate the capacity of the global gene pool to respond to our use of antibiotics [D'Costa *et al.* and (*5*)].

ROGER W. PICKUP* AND GLENN RHODES

Centre for Ecology and Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, Lancashire LA1 4AP, UK.

*To whom correspondence should be addressed. E-mail: Roger@ceh.ac.uk

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## TECHNICAL COMMENT ABSTRACTS

### Comment on "Reconstructing Past Climate from Noisy Data"

**Eugene R. Wahl, David M. Ritson, Caspar M. Ammann**

von Storch *et al.* (Reports, 22 October 2004, p. 679) criticized the ability of the "hockey stick" climate field reconstruction method to yield realistic estimates of past variation in Northern Hemisphere temperature. However, their conclusion was based on incorrect implementation of the reconstruction procedure. Calibration was performed using detrended data, thus artificially removing a large fraction of the physical response to radiative forcing.

Full text at www.sciencemag.org/cgi/content/full/312/5773/529b

### Response to Comment on "Reconstructing Past Climate from Noisy Data"

**Hans von Storch, Eduardo Zorita, Julie M. Jones, Fidel González-Rouco, Simon F. B. Tett**

We implemented a proxy-based method for reconstructing temperatures in the past millennium in simulations with two climate models using the pseudoproxy approach. We show results for detrended and nondetrended calibration using white-noise and red-noise pseudoproxies with realistic noise levels. In all cases, the method underestimates the low-frequency variability of the simulated Northern Hemisphere temperature.

Full text at www.sciencemag.org/cgi/content/full/312/5773/529c

EVOLUTIONARY GENETICS

# Broken Cogs or Strategic Agents?

**Peter Hammerstein and Edward H. Hagen**

Thirty years ago, Richard Dawkins shook the scientific community with provocative reflections on the role of genes in evolution (*1*). He drew our attention to the fact that "selfish genes" boosting their own transmission at the expense of the organism in which they act may spread in a population despite their harmful effects. Evolutionary biologists have subsequently debated how frequently such genes actually occur and how they have shaped animals, plants, and fungi.

*Genes in Conflict*, by evolutionary geneticist Austin Burt (Imperial College London) and biologist Robert Trivers (Rutgers University), is the first book to review the vast empirical literature on selfish genetic elements. It reveals how widespread these elements are in nature, what evolutionary effects they have had on fundamental aspects of the genetic system itself (such as its size, organization, and degree of recombination), and how they influence reproduction, development, and behavior. While enthusiastically addressing the ever-accelerating advance of genetic conflict studies, the authors also take care to identify many open questions. Their fascinating and comprehensive book provides a gold mine for anyone entering the field.

**Genes in Conflict**
The Biology of Selfish Genetic Elements

***by Austin Burt and Robert Trivers***

Harvard University Press, Cambridge, MA, 2006. 620 pp. $35, £21.95. ISBN 0-674-01713-7.

Selfish genetic elements are more than just genes; they also include stretches of noncoding DNA, fragments of chromosomes, etc. It is helpful to view these elements as agents "acting in their own interest" and "being in conflict" with other parts of the genome, as the book's title suggests. Using this heuristic, it is "in the interest" of some genes, for example, to protect chromosomes from damage, whereas it is in the interest of others to break chromosomes to get themselves replicated by the repair process or to jump across the DNA replication complex and thus get replicated more than once as the complex travels along a chromosome. The book beautifully demonstrates the power of the genes-as-agents perspective, which has opened our eyes to arms races within the genome and to limits imposed on the unity of the organism.

Theoretical biologists, however, would add a word of caution. Insights gained from this approach must be carefully checked using equations that describe how genetic elements invade a population and compete with one another. The dynamics of selfish elements in host populations is more complex than a simple heuristic can capture, as the following example shows. In the wasp *Nasonia vitripennis*, a selfish supernumerary chromosome, called PSR (for paternal sex ratio), disables the transmission of all regular chromosomes from father to offspring. Infected males thus contribute genetically no more than the selfish element to an offspring. This is in the interest of PSR because—skipping a little biology here—it also changes the offspring's sex from female to male, and males are better than females at passing PSR to offspring. Is this effect sufficient to understand the spread of PSR? It is evidence of the high quality of *Genes in Conflict* that the book pays attention to mathematical results, as in its short verbal account of an analysis done by Jack Werren and Leo Beukeboom (*2*). That analysis revealed a surprise: PSR's trick only leads to invasion when there is a female-biased sex ratio. This insight comes from population biology rather than from anything resembling decision theory or game theory—the mathematical formalizations of strategic agents. Nevertheless, without the genes-as-agents heuristic, much of the material covered in the book would never have been understood, because mathematics is often better for scrutinizing ideas than for developing them.

Many readers will appreciate that in addition to presenting the state of the art, the book also includes a concise history of the long struggle to understand selfish elements. Exactly 100 years ago, for example, Carl Correns discovered the phenomenon of cytoplasmic male sterility (the suppression of male function by cytoplasmic genes) in plants (*3*). Thirty-five years passed before Dan Lewis realized that this effect of cytoplasmic genes conflicts with the interests of genes in the cell's nucleus (*4*). Another 40 years elapsed before conflict between the nucleus and cell organelles received more general attention from Leda Cosmides and John Tooby (*5*). Today, a quarter-century later, several examples of intracellular symbionts that kill males or prevent mothers from producing them have either been discovered or better understood as selfish genetic elements. The current rapid rate of progress in genetic conflict studies probably stems from the development of molecular and cytogenetic techniques plus the much closer links between empirical and conceptual advances than for most of the 20th century.

Given its primary goal, *Genes in Conflict* must present a rich variety of phenomena, but doing so risks drowning the reader in facts. To prevent this, the authors have written each chapter so that it stands alone (at the cost of some redundancy). They have also relied on a commendable conceptual clarity to guide readers through the jungle of details. In particular, Burt and Trivers acknowledge at the outset that most genes are cooperative, restricting the notion of selfish genes to those few genes that increase their own reproduction at the expense of the organism as a whole.

Are genes best conceptualized as agents? Or as cogs in a complex machine that cause problems when broken? Although molecular and population biology typically emphasize the latter view, Burt and Trivers convince us that we would be missing something important if the strategic gene were ignored. Nonetheless, we believe at least two criteria must be satisfied for a strategic framework to be productively applied to selfish genetic elements. First, such elements should show evidence of complex design that reflects multiple independent strategically advantageous modifications. (If the only examples were elements that hijack existing complex machinery via a single modification, the strategic framework would be overkill.) Second, there should be evidence of evolutionary moves and countermoves between selfish elements and other parts of the genome. The book's plethora of facts includes support for both criteria. Many selfish elements, for example, show directional movement, tissue specificity, or time-dependent effects—prima facie evidence for complex design. And the widespread occurrence of suppressors clearly indicates countermoves.

As much as we appreciate the strategic viewpoint, it presents some obvious pitfalls. To name one, just as our visual system readily interprets three dots as a face, our hypersocial brains might be seeing strategies where none exist. As the authors admit, the molecular mechanisms employed by selfish elements are in many cases unknown, which makes it difficult to interpret important aspects of their evolution. In providing such a superb review, Burt and Trivers have laid the foundation for further exploration of these important conceptual issues.

The reviewers are at the Institute for Theoretical Biology, Humboldt University, Invalidenstrasse 43, 10115 Berlin, Germany. E-mail: p.hammerstein@biologie.hu-berlin.de; e.hagen@biologie.hu-berlin.de

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10.1126/science.1125754

ECONOMICS

# Nothing Succeeds Like Failure

**Sam Kean**

There are two conclusions, neither of them satisfying, one can draw from Paul Ormerod's thesis that the extinction patterns of biological species and business firms not only look similar, but are undergirded by the same "law of failure." On the one hand, Ormerod points out in *Why Most Things Fail*, perhaps both corporations and species plan their evolution. But this amounts to saying that ringworms can tinker with their DNA as easily as managers adjust their capital projections and budgets. The other, and much more likely, conclusion is that corporate evolution is as random as biological evolution. Things aren't quite so hopeless, Ormerod writes, but "firms are no more capable of planning and securing favourable outcomes with their changes of strategy than sunflowers are capable of deciding to grow feet better to follow the sun."

**Why Most Things Fail**
Evolution, Extinction and Economics

***by Paul Ormerod***

Pantheon, New York, 2006. 269 pp. $24.95. ISBN 0-375-42405-9. Faber and Faber, London, 2005. £12.99. ISBN 0-571-22012-6. Paper, 2006. £8.99. ISBN 0-571-22013-4.

It's not that firms don't try to grow feet. But the world is so multifarious, so complex, that planning does little good. Over one hundred years, a certain number of firms will inexorably disappear, just as a certain number of species will over one million. Having assets helps put off extinction—a billion in cash, or huge teeth. Nonetheless, external shocks and internal changes will eventually render most beasts and businesses unable to cope, and predicting which causes will be decisive is extremely difficult.

Ormerod (an economic forecaster and founder of the British consulting firm Volterra) touts the book as an explanation of failure, saying that scientists (including economists) spend too much time studying success. Failure, after all, is much more common. Yet, the author is more concerned with uncertainty. His previous book, *Butterfly Economics* (*1*), took its title from the chaos-theory chestnut, "a butterfly flaps its wings in China…." His points about extinction are thought-provoking, but uncertainty is always in the front seat. Failure is an afterthought:

> the future remains covered in a deep veil to all. Species, people, firms, governments are all complex entities that must survive in dynamic environments which evolve over time. Their ability to understand such limits is inherently limited.
>
> …limits can no more be overcome by smarter analysis than we are able to break binding physical constraints, such as our inability to travel faster than the speed of light. This is why things fail.

As in this passage, Ormerod writes lucidly and cleanly, and he only occasionally gives in to lyrical impulses. Other pop science writing seizes the reader with catchy leads and sweeping rhetoric. Ormerod's opening sentence reads, "The period from around 1880 to 1910 saw the emergence of radically different ways of organizing and carrying out economic activity." That "radically" doesn't fool anyone.

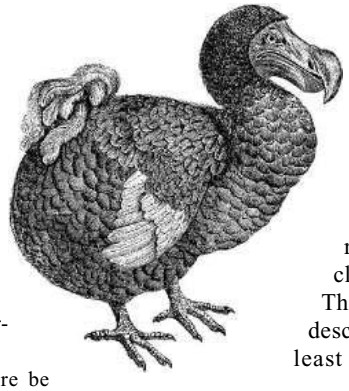
Yet the book does not lack digressions. Ormerod discusses *MacBeth*, *The Sopranos*, and the near-fatality of the young Microsoft corporation. Most sidebars are apt, although it seems that the author has never actually seen *The Price Is Right*, only read economics papers about it. My one complaint is that, however much Uncle Joe deserves it, Ormerod should have stopped pointing out the failures of central planning in Stalin's USSR and elaborated on his mistrust of central planning in the European Union.

Similarly wide-ranging are the scientific tools Ormerod incorporates. He talks of half-lives and the tension between dominant individuals and the propagation of species. He uses something like social-network theory to form computer simulations where each "agent" has predefined connections to all other agents, and one extinction can wipe out dozens of friends.

Ormerod is especially enamored of game theory and cellular autonoma, both of which reinforce the idea that outcomes are often impossible to predict from initial conditions. Most of the dozen figures in the book are merely functional, but the one illustrating Thomas Schelling's model for geographic segregation is revealing: the only initial rule was that "red" and "blue" cells exhibit slight preferences for their own "race" and will move around accordingly. After a few generations, the initially mottled picture looks like a voting district ripe for gerrymandering, with clumps of dark and light patches. The many, many simulations he describes help Ormerod prove (at least in silico) that changes in internal relationships, whether between species or firms, help explain failure more accurately than do occasional external disruptions, like meteors or bear markets. It's not them, it's us.

Indeed, anyone with a slight libertarian bent will find fodder in *Why Most Things Fail*. Whether dissecting race, class, or economics, Ormerod is suspicious that any complex policy will work. Still, "[t]his is not to say that all public policy necessarily ends in failure," he writes. "Almost at random, some will succeed." Not that governments do worse than businesses: "To repeat a key phrase which needs to be hard-wired into the brain of every decision-maker, whether in the public or private sector, intent is not the same as outcome."

Although Ormerod's assertions are intriguing, it's hard to know how far to trust them. The paleontology that establishes extinction data has gaps and is hard to quantify. Information about corporate failure is sparse, too. A full-fledged empiricist, Ormerod can manipulate data marvelously, and the two types of extinctions do follow strikingly similar statistical patterns—"power laws." But whether this adds up to an "iron law of failure" is a different matter. Ormerod's outcome may not prove the same as his intent.




**Three iconic failures.** The dodo, Sony's Betamax videotape, and Ford's 1958 Edsel.

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The reviewer was a 2005 AAAS Mass Media Science and Engineering Fellow. E-mail: samkean@gmail.com

**Reference**

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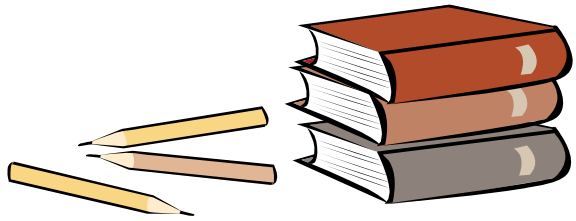
COMPUTER SIMULATIONS

# Technological Advances in Inquiry Learning



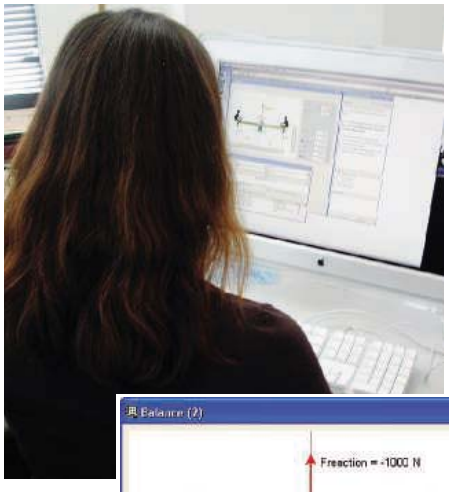
**Ton de Jong**

Computer simulations enhance inquiry-based learning—in which students actively discover information—by allowing scientific discovery within a realistic setting.

The promise offered by inquiry learning is tempered by the problems students typically experience when using this approach. Fortunately, integrating supportive cognitive tools with computer simulations may provide a solution.

## Learning by Inquiry

Studies of young students' knowledge and skills indicate that many students in large parts of the world are not optimally prepared for the requirements of society and the workplace (*1*). To meet this challenge, curricula should be designed to help students learn how to regulate their own learning, how to continue to gain new knowledge, and how to update their existing knowledge.



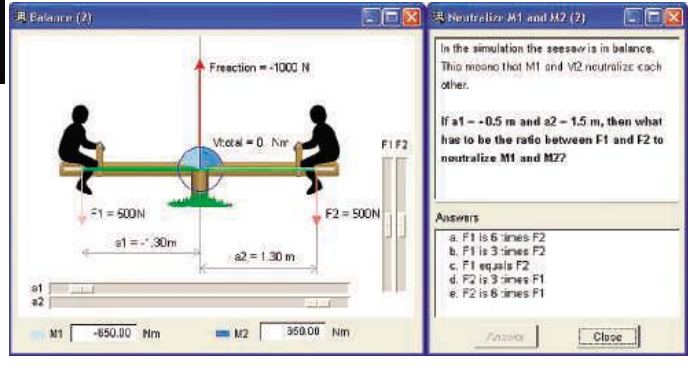
Inquiry learning is defined as "an approach to learning that involves a process of exploring the natural or material world, and that leads to asking questions, making discoveries, and rigorously testing those discoveries in the search for new understanding" (*2*). This means that students adopt a scientific approach and make their own discoveries; they generate knowledge by activating and restructuring knowledge schemata (*3*). Inquiry learning environments also ask students to take initiative in the learning process and can be offered in a naturally collaborative setting with realistic material.

The idea of inquiry, or discovery, as a learning approach has a long history (*4*, *5*). Now, technological developments such as computer simulations can implement more effective inquiry learning. Using simulations to model a phenomenon or process, students can perform experiments by changing variables (such as resistances in an electrical circuit) and then observe the effects of their changes (e.g., the current). In this way, students (re-)discover the properties of the underlying model (Ohm's law).

## The Inquiry Process

Inquiry learning mimics authentic inquiry. [There are some exceptions, such as the origin of the research question, the number of (known) variables, and the presence of flaws in data (*6*).] Because they are closely related, they share the following constitutive cognitive processes (*7*): orientation (identification of variables and relations); hypothesis generation (formulation of a statement or a set of statements, perhaps as a model); experimentation (changing variable values, making predictions, and interpreting outcomes); reaching conclusions (on the validity of the hypothesis); evaluation (reflection on the learning process and the acquired knowledge); planning (outlining a schedule for the inquiry process); and monitoring (maintaining an overview of the inquiry process and the developing knowledge).

However, research indicates that, overall, students have substantial problems with all of the inquiry processes listed above (*8*). Students have difficulty choosing the right variables to work with, they find it difficult to state testable hypotheses, and they do not necessarily draw the correct conclusions from experiments. They may have difficulty linking experimental data and hypotheses, because their pre-existing ideas tend to persist even when they are confronted with data that contradict those ideas (*9*). Students also struggle with basic experimental processes. They find it difficult to translate theoretical variables from their hypothesis into manipulable and observable variables in the experiment (*10*); they design ineffective experiments, for example, by varying too many variables at one time (*11*); they may use an "engineering approach," where they try to achieve a certain state in the simulation instead of trying to test a hypothesis (*12*); they fail to make predictions; and they make mistakes when interpreting data (*13*). Students also tend to do only short-term planning and do not adequately monitor what they have done (*14*).




**A SimQuest application on the physics of moments.** Students can change the two forces acting on the people (F) and the distances to the center of the seesaw (a) and discover the effect on the moment (M).

## Supporting the Inquiry Process

Research in inquiry learning currently focuses on finding scaffolds or cognitive tools that help to alleviate these problems and produce effective and efficient learning situations. Computer environments can integrate these cognitive tools with the simulation. Examples of cognitive tools are assignments (exercises that set the simulation in the appropriate state); explanations and background information; monitoring tools (to help students keep track of their experiments); hypothesis scratchpads (software tools to create hypotheses from predefined variables and relations); predefined hypotheses; experimentation hints (such as "vary one thing at a time " or "try extreme values"); process coordinators (which guide the students through the complete inquiry cycle); and planning tools. Overviews can be found in (*7*) and (*15*); examples of integrated inquiry systems are

The author is at the Faculty of Behavioral Sciences, University of Twente, Enschede 7500AE, Netherlands. E-mail: a.j.m.dejong@utwente.nl

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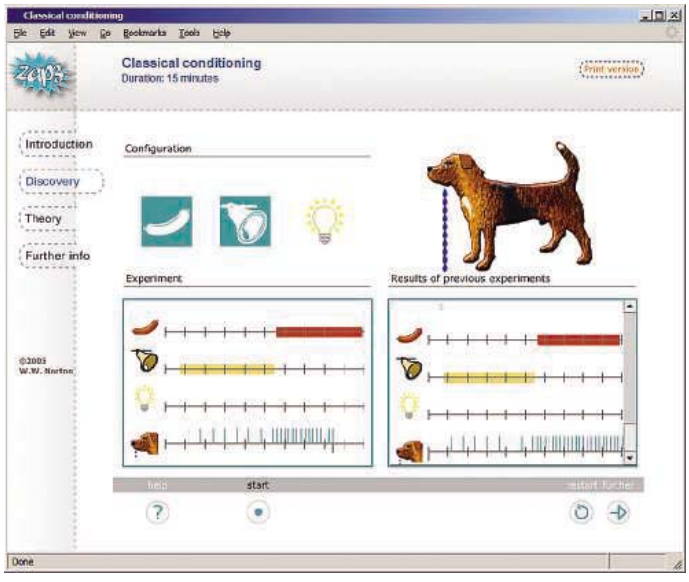
SimQuest applications (*16*), Co-Lab (*17*), GenScope (*18*), and Inquiry Island (*19*).

One example from a SimQuest application explores the physics of moments (see the figure on page 532) (*20*). Support is offered in the form of an assignment that asks students to explore the balance of the seesaw by changing variables. Another available aid is a hypothesis scratchpad that lets students build expressions from variables (e.g., force F1, distance a1, and moment M1) and relations (e.g., increases) to create testable hypotheses (e.g., if F1 increases, then M1 increases).

Most experimental evaluations of cognitive tools offer different configurations of learning environments to different experimental groups. Effects measured include the acquisition of conceptual knowledge, procedural knowledge, and/or inquiry skills. Often the learning process can be analyzed from log files that track the behavior of students in the learning environment and/or data from students who are requested to think aloud during learning. The most effective learning results are found with tools that structure the learning process, provide students with predefined hypotheses and background information, help students plan (e.g., by providing a sequence of assignments), or give hints for efficient experimentation (*7*, *15*, *21*). For example, students offered simulations and assignments performed better in tests of intuitive knowledge of the physics of oscillation (*22*). Also, biology students who received prompts on experimental strategies outperformed in tests those who received other prompts or no prompts at all (*23*).




**A simulation of psychological conditioning.** Students can perform multiple trials and can offer the dog a sausage, ring the bell, and/or light the lamp and then observe the salivation of the dog. In this way, they explore principles of conditioning, second-order conditioning, and extinction.

## The Road Ahead

Unguided inquiry is generally found to be an ineffective way of learning (*24*). Reviewing classical research in three areas of learning—problem-solving rules, conservation strategies, and programming concepts—Mayer (*3*) concluded that guided discovery learning is effective. These guided inquiry environments are starting to enter educational practice, especially for ages 14 and up, and large-scale evaluations are promising (*18*). Mostly physical science topics have been tested, but inquiry environments have been used in other areas. In psychology, for instance, simulations have modeled Pavlovian (classical) conditioning, where an organism learns to relate one event to another previously unrelated event (*25*, *26*) (see the figure below).

A number of research issues still lie ahead. First, the introduction of cognitive tools may lead to overly complex learning environments that hinder learning by requiring too much working memory capacity. Ways to reduce this extraneous cognitive load, such as by integrating representations (*27*), are being investigated. Another challenge lies in adapting the learning environment to respond not only to differences between learners but also to the developing knowledge and skills of an individual learner. Learning environments could use "fading," in which cognitive tools gradually disappear so that the learner can ultimately take over the learning process. Automating this would need an adequate cognitive diagnosis of both a student's learning process and developing knowledge and might be based on the log files of the student's interactions with the system (*28*). A further challenge is to find ways to combine collaborative learning and inquiry learning (*17*, *29*). Specific tools to structure the collaboration and sharing of (intermediate) models between students are only now being developed. Students may also be offered the opportunity to create informal models (*17*). Such a facility helps them to articulate intuitive knowledge and at the same time gives them a specific task to complete.

Sound curricula combine different forms of tuition, both inquiry learning and direct instruction. Inquiry learning may be more effective in acquiring intuitive, deep, conceptual knowledge; direct instruction and practice can be used for more factual and procedural knowledge. Ultimately, we want students to gain a well-organized knowledge base that allows them to reason and solve problems in the workplace and in academic settings. Finding the right balance between inquiry learning and direct instruction, therefore, is a major challenge.

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BIOPHYSICS

# Lonely Voltage Sensor Seeks Protons for Permeation

The proton channel that ushers $H^+$ ions across cell membranes to control cellular pH resembles an isolated voltage-sensing domain with no apparent pore.

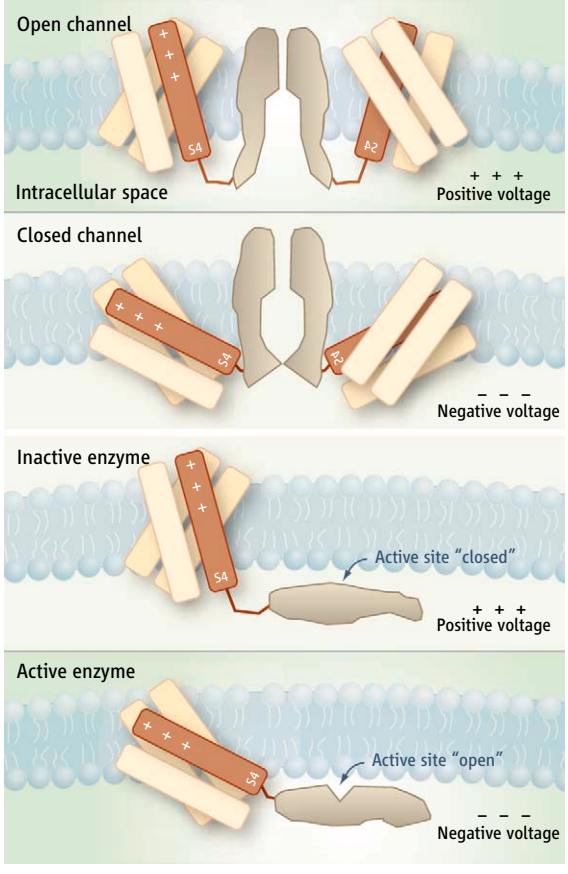
**Christopher Miller**

The file-cabinet in my office houses detritus of a pre-electronic past: manila folders stuffed with long-neglected reprints from a time when ion channels were known only by the electrical properties they display in electrophysiological experiments. Today, nearly all these ion channels are also known as proper membrane proteins, their sequences assigned to molecular families, the detailed workings of many well understood. Over the past 25 years, these ion channels fell one by one to the awesome powers of recombinant DNA, membrane biochemistry, and in a few cases x-ray crystallography: channels gated by voltage, neurotransmitters, or intracellular ligands; channels modulated by G proteins, $Ca^{2+}$, phosphorylation, or all of these together; channels sensitive to heat and cold, jalapeno and menthol, mechanical stretch, even light.

But in this all-electronic age, there is one manila folder that I still dip into. It is labeled "Funny Little Channels," and its reprints deal with a stubbornly defiant hold-out against molecular identification: the voltage-dependent proton channel originally discovered in snail neurons (*1*) and meticulously characterized in mammalian cells by DeCoursey (*2*, *3*). Cells use this channel for proton extrusion in response to membrane voltage changes, in contexts as diverse as bacterial killing by macrophages and bone resorption by osteoclasts (*2*). The channel's quirky functional personality seems to place it outside all known ion channel families, but the gene encoding this protein has eluded discovery. Two recent papers (*4*, *5*) unveil the molecular identity of the voltage-gated proton channel. The surprising results match the channel's functional idiosyncrasies and open the door to a deeper understanding of voltage sensing in membrane proteins.

To appreciate the force of the discovery, it is useful to review a group of ion channels called the S4 family. These proteins—gated transmembrane pores specific for $Na^+$, $K^+$, or $Ca^{2+}$—fulfill a range of biological needs, the best known of which is the charge flow required to generate electrical signals in nerve cells. S4 proteins sense and respond to transmembrane voltage changes. For example, at a typical resting potential of –70 mV within the cell, $Na^+$ channels are closed, but when the voltage moves positive, they open and trigger electrical activity.

This conformational switching can be understood by looking at the structures of S4 proteins (see the figure, top panels). In all these proteins, a membrane-spanning module is repeated four times around the pore axis. Each module consists of six transmembrane helices. The last two helices, S5 and S6, form the ion-selective pore in the center. The first four, S1 to S4, fold together on the pore periphery into a separate voltage-sensing domain (VSD), a molecular voltmeter that tells the pore when to open or close.




**Voltage-sensing domains in two contexts. (Top two panels)** A typical voltage-gated $K^+$ channel, with voltage-sensing domain (yellow and brown rods) attached to a gated pore. **(Bottom two panels)** Voltage-sensing domain attached to lipid phosphatase to form a voltage-gated enzyme. [Adapted from (*7*)]

The physical basis of this key action resides in the unusual sequence of helix S4. This otherwise hydrophobic sequence is peppered with positively charged residues (usually arginines separated by three or four residues). At negative voltage, the helix is electrostatically drawn toward the intracellular side of the membrane; when the voltage becomes positive, the helix is pushed outward. These movements tug on the pore domain to close or open the channel. The recent crystal structure of an open S4 channel (*6*) reveals the four-leaf-clover arrangement of VSDs around the central pore.

VSDs were long thought to occur only in the S4 family. But in 2005, in a sea-squirt genome project, Okamura and co-workers discovered a VSD-containing protein that is not part of an ion channel (*7*). Instead, the VSD, complete with an arginine-laden S4 helix, is attached to the catalytic domain of a lipid phosphatase (see the figure, bottom panels). This protein is the first known voltage-controlled enzyme. Okamura and co-workers then used the sea-squirt VSD sequence to find mammalian homologs, but no such phosphatases were pulled out. Instead, the mouse genome coughed up the VSD-containing protein described by Sasaki *et al.* on page 589 of this issue (*4*). Using a slightly different approach, Ramsey *et al.* obtained a human homolog (*5*). The big surprise is that this VSD is not attached to anything at all: The protein is a free-floating VSD.

To find out what the VSD does, both groups used a heterologous system (one that does not normally express the protein) to produce the protein for electrophysiological analysis. Robust voltage-dependent proton currents were observed, with properties similar to those that appear naturally in many cell types. Similarities in the heterologous versus natural systems include gating that depends on voltage and transmembrane pH gradient, high temperature sensitivity, and inhibition by $Zn^{2+}$. Cherny and DeCoursey (*8*) had predicted from detailed mechanistic analysis that the voltage-dependent $H^+$ channel would carry a conserved pair of extracellular-facing histidines mediating $Zn^{2+}$ inhibition; such a pair is observed in (*4*, *5*). Moreover, mutation of the S4 arginines produces changes in gating kinetics and softening of voltage dependence, as expected for a VSD. These correspondences led both groups to con-

The author is in the Department of Biochemistry, Howard Hughes Medical Institute, Brandeis University, Waltham, MA 02454, USA. E-mail: cmiller@brandeis.edu

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clude that the VSD alone forms the classical voltage-gated proton channel, the founding member of the new $H_v$ channel family (*4*, *5*).

That is a satisfying conclusion. But here is a gentle caution from someone once burned by applying exactly the same criteria—similarity of electrophysiological properties, alteration of functional behavior by mutation, appropriate tissue distribution—to infer that a quirky $K^+$ channel protein, minK, is identical to a particular $K^+$ current of human cardiac tissue (*9*). That conclusion was later shown to be wrong: minK is an auxiliary subunit of a conventional $K^+$ channel (*10*). Nevertheless, the idea of an ion channel formed by a solitary VSD is so chemically intriguing, biologically rich, and aesthetically pleasing that I will refrain from demanding the tight proof normally expected for family founders: functional reconstitution of the purified protein.

With the arrival of the $H_v$ family, I can almost hear the patch-pipettes pulling and PCR tubes popping as biophysicists rush to attack new questions. Why does the channel not have a pore domain? Maybe because protons, unlike metal cations, do not need an aqueous pathway to move through proteins (*3*). Where do the protons go? Probably not along the S4 helix itself, despite the fact that a proton leak can be engineered into a $K^+$ channel's S4 helix (*11*). How many VSDs associate to form the channel? Two or more, surely, because at least six charges move across the membrane upon opening (*3*). What does the VSD look like? Probably similar to the known structure of an isolated, though nonfunctional, VSD of a $K^+$ channel (*12*). And how will knocking out this gene affect the health of a mouse?

I reckon that I will be saving the many future papers addressing these questions as PDFs.


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PLANETARY SCIENCE

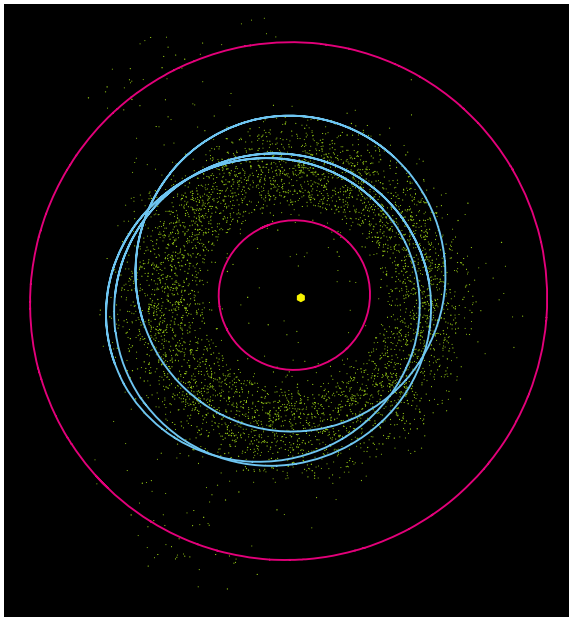
# Ice Among the Rocks

Two groups of comets are known: those with orbital periods of hundreds of years or greater, and those with decade-long periods. A third class appears to be orbiting within the asteroid belt.

**Alan Fitzsimmons**

Astronomers have known for more than two centuries that comets can be split into two groups as defined by their orbits about the Sun. Long-period comets, so named because they have orbital periods of more than 200 years, originate from the Oort Cloud of comets surrounding the Sun and stretching at least 10% of the way to the nearest star (*1*). The second group are known as the Jupiter-family comets, with orbital periods near 20 years, whose dynamical evolution is controlled by gravitational encounters with the giant planet. Theoretical work pinpointed the source of this second group to a comet belt beyond the planet Neptune (*2*–*4*); this was dramatically proven by the discovery of the first such object in 1992 (*5*). Now, in a report on page 561 of this issue, Hsieh and Jewitt (*6*) have issued a shock to the system by demonstrating the existence of a third dynamical class of comets, orbiting much closer to the Sun and lying entirely within the main asteroid belt.

The story starts in 1996 with the discovery that an asteroid first seen 17 years earlier was in fact a comet, henceforth named 133P/Elst-Pizzaro (*7*). Observationally, all but the largest asteroids are optically unresolved and appear as point sources, whereas active comets are recognizable when near the Sun from the surrounding atmosphere of sublimated ices and dust particles. Each year, several objects classified as asteroids but lying in elongated comet-like orbits are found to exhibit a coma and/or tail and hence are reclassified as comets. The surprising fact about 133P/Elst-Pizarro was that its orbit was unlike that of any other comet, as it lay completely within the asteroid belt between Mars and Jupiter. Another comet on a similar orbit was discovered late last year, and Hsieh and Jewitt report finding a third in a dedicated survey for such objects. All three objects are relatively stable against strong gravitational perturbations from Jupiter, which implies that they exist where they formed.



**Closer to home.** Orbits of the three main-belt comets discussed by Hsieh and Jewitt (blue) and the planets Mars and Jupiter (red). The green points are the first 5000 asteroids numbered, showing the position of the main asteroid belt.

Hsieh and Jewitt show that the detected atmosphere of dust particles cannot be caused by weak processes such as electrostatic levitation, nor can it be the debris cloud from an impact by a smaller body, and hence it must result from the steady sublimation of ices as with other comets. In most walks of life, two is company but three is a crowd, and there is no escaping the recognition that we now have a third dynamical class of active comets identified in the solar system, which Hsieh and Jewitt have labeled main-belt comets.

Like all good discoveries, this throws up a number of questions. Perhaps the most important is how they can exist in the first place. Comets are ephemeral bodies, as each time they pass the Sun they lose a small fraction of their mass via sublimation of the surface ices. For example, the lifetime of Mark Twain's nemesis, Halley's comet, has been estimated as less than 100,000 years (*8*). The comets we see today disappear on these time scales, to be replenished by new comets from the Oort Cloud and the trans-Neptunian reservoirs. But the main-belt comets are still in their source regions, where continuous solar heating would have seen them vanish very soon after formation.

The author is at the Astrophysics Research Center, School of Mathematics and Physics, Queen's University Belfast, Belfast BT7 1NN, Northern Ireland, UK. E-mail: a.fitzsimmons@qub.ac.uk

Hsieh and Jewitt believe that the likely answer for main-belt comets is that they have suffered a small collision in the recent past, which has exposed subsurface ices to solar heating, and that these ices may sublimate on and off for at least several years before exhaustion. This is supported by observations showing that 133P/Elst-Pizarro has been only sporadically active over the past decade (*9*, *10*). Given that last year's spectacular Deep Impact mission (*11*) did not result in a new activity site on a normal Jupiter-family comet, our demonstrable lack of knowledge of how sublimation sites are activated implies that a better estimate of the sublimation lifetime is unlikely in the near future.

It is also unclear how many main-belt comets may exist. Hsieh and Jewitt estimate that there may be as many as 150 currently detectable in this new population, although they caution that true numbers will require a much larger systematic survey. The excitement for planetary scientists is that we now have a new direction in which to study the composition of the solar system. Current theories predict that both Jupiter-family comets and long-period comets formed in the outer solar system beyond Jupiter and were scattered into their present orbits via various gravitational perturbations. The main-belt comets are relatively immune to such effects and should be pretty close to their birthplace. Hence, by studying the ices in these comets, astronomers could look for changes in the ice composition in the protoplanetary disk. This makes main-belt comets a prime target for future space missions, but it may be possible to start such studies using the next generation of optical, infrared, and submillimeter telescopes currently being built or planned.

At the same time, Hsieh and Jewitt note that the outer asteroid belt has been proposed as a source of the water deposited on Earth after the end of the planet-building phase. This work should spur a closer assessment of recent dynamical models predicting delivery of large numbers of objects from this region into near-Earth space (*12*). It is interesting that many astronomers have pursued comets to greater and greater distances in their pursuit of understanding the evolution of comets and the early history of the solar system. All this time, it would have also been worthwhile to look a little closer to home.

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10.1126/science.1126896

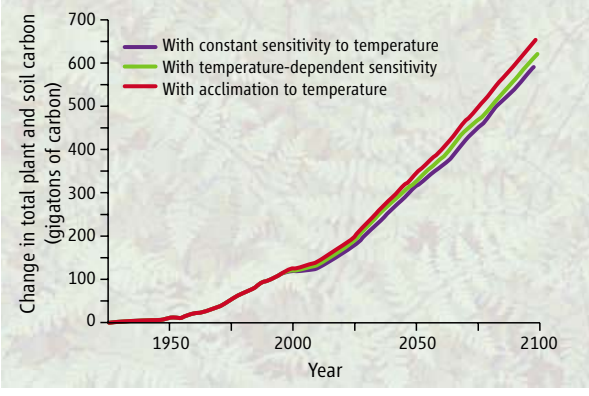
ATMOSPHERE

# Plant Respiration in a Warmer World

Acclimation of plants to higher temperatures may reduce the extra warming caused by increased plant respiration in a future warmer world.

**Anthony W. King, Carla A. Gunderson, Wilfred M. Post, David J. Weston, Stan D. Wullschleger**

Plants release carbon dioxide as they metabolize carbon substrates for biosynthesis and maintenance of the biochemical machinery of life (*1*, *2*). This respiratory process globally transfers about 60 gigatons of carbon each year to the atmosphere (*3*). It has been predicted that plant respiration, and leaf respiration in particular, will increase in a future warmer world. But are these predictions consistent with observations from modern experimental studies?




**The effect of respiration.** Cumulative change in global total terrestrial biosphere carbon simulated by the GTEC 2.0 model, using different temperature dependencies for leaf respiration. See the supporting online material.

Numerous studies have shown that respiration increases in response to an increase in temperature (*4*, *5*). Higher plant respiration at warmer global temperatures would release more $CO_2$ to the atmosphere, resulting in lower net ecosystem carbon uptake, even higher atmospheric $CO_2$ concentrations, and consequently more warming. Incorporating biotic feedbacks like this in coupled climate-carbon models results in an additional increase of simulated mean annual land-surface temperatures of as much as 2.5ºC by 2100 (*6*, *7*).

However, many studies have shown that the increase in plant respiration in response to an increase in temperature is a short-term, largely transient response that is observed when plants grown at a controlled temperature are experimentally exposed to warmer temperatures. In the longer term, plant respiration may acclimate to warmer temperatures. Plants experimentally grown at higher temperatures often respire at nearly the same rate as plants grown at cooler temperatures, even though a short-term warming of either set of plants would produce a typical exponential response to temperature (*8*–*10*). In addition, plants from warmer climates often show a much-reduced sensitivity to temperature change when compared to plants from cooler climatic regions (*11*).The biochemical basis for acclimation is not yet known. Mechanistic synthesis, understanding, and modeling are thus problematic, and a mechanistic representation of the acclimation of plant respiration to temperature is generally absent from climate change analyses and carbon cycle models. An increasing number of physiological studies do, however, support the conclusion that the long-term response of respiration to temperature may be quite different from the more commonly measured and short-term response.

Acclimation of respiration to elevated temperatures has clear implications for predictions and expectations of higher plant respiration in a warmer world. For example, reduced sensitivity of respiration to temperature increase could reduce the magnitude of the positive feedback between climate and the carbon cycle in a warming world. Yet, though most coupled

The authors are in the Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA. E-mail: kingaw@ornl.gov

climate-carbon models include an increase in leaf and plant respiration in response to elevated temperature, none in the C⁴MIP climate-carbon model intercomparison (*12*) and no others to our knowledge include an explicit time-dependent acclimation of plant respiration to increasing temperatures. Some differentiate among vegetation types, such that the response to warming temperatures of tropical vegetation is smaller than that of boreal vegetation, for example. And in some, the sensitivity to temperature depends on temperature, so that respiration increases more slowly with warming at higher temperatures than at cooler temperatures. But even these models do not include the time-dependent acclimation to a change in temperature within a few days observed in experiments (*9*).

Recent work with an ecosystem-scale model showed how acclimation of respiration to changing temperature could have a substantial effect on rates of aboveground net primary production (*13*). To explore this issue further, we have investigated the influence of temperature acclimation of leaf respiration on simulated carbon dynamics and climate-carbon feedbacks at both the local ecosystem scale and the global scale. Plant parts other than leaves are also likely to acclimate to warmer temperatures, but because more is known about leaves, we have limited our analysis to leaf respiration.

The figure compares the changes from 1930 to 2100 of total carbon stored globally in plants and soils simulated by a global terrestrial ecosystem model, GTEC 2.0 (*14*), with and without acclimation of leaf respiration. The standard version of the model uses a constant sensitivity to temperature; the sensitivity to temperature varies with vegetation type, but does not change with time or temperature. As did Wythers *et al.* (*13*) in their ecosystem-scale model, we performed two further model runs but at the global scale, one with a temperature-dependent sensitivity to temperature (the increase in respiration with increase in temperature is less at warmer temperature, and respiration actually declines with even further warming) and one with an empirical representation of the acclimation of leaf respiration to temperature change based on observations from plant-warming experiments (*14*).

The simulated increase in total carbon stored globally in plants and soil is smallest with the constant sensitivity to temperature, slightly higher with temperature-dependent sensitivity, and largest with acclimation (see the figure). With acclimation (even the partial acclimation we model), leaf respiration at the higher temperatures at the end of the 21st century is reduced, and more carbon is stored in plants and soils. All other things being equal, as they are in our simulations, more carbon stored in plants and soils corresponds to less carbon released to the atmosphere in response to climate change, and a weaker positive feedback between carbon and climate and a weaker amplification of additional warming.

Thus, acclimation of leaf respiration (a known phenomenon, but one not normally included in coupled climate-carbon models) has the potential to reduce the strength of the positive feedback between climate and carbon commonly found in coupled climate-carbon simulations. The effect in the reported simulations is small compared with differences among models (*12*), and our sensitivity analysis (*14*) uses a single empirical representation of leaf acclimation drawn from a limited set of experiments. Nevertheless, the influence of acclimation of leaf respiration to temperature is of sufficient magnitude in our analysis to suggest that it should be incorporated into plant, ecosystem, and coupled climate-carbon simulations.

There is also a need to better understand the control of respiration itself. The development, testing, and adoption of a mechanistic and biochemical model of plant respiration are needed. To more reliably project plant respiration and climate-carbon feedbacks in a future climate, this modeling must incorporate response to temperature, including acclimation, at time scales from minutes to years.

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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/312/5773/536/DC1
SOM Text
References

10.1126/science.1114166

EVOLUTION

# Size Does Not Matter for Mitochondrial DNA

**Adam Eyre-Walker**

That large populations harbor more genetic diversity than small ones holds true for nuclear genomes, but may not apply to mitochondrial DNA. If so, the use of mitochondrial DNA as a standard for genetic diversity may not be appropriate.

On page 570 of this issue, Bazin *et al.* (*1*) test one of the most basic predictions of population genetics: that species with large population sizes should have more genetic diversity than species with small population sizes. They find that this prediction, as expected, is upheld for diversity in nuclear genes, but that there is no correspondence between population size and genetic diversity for mitochondrial genes.

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The author is at the National Evolutionary Synthesis Center, Durham, NC 27705, USA, and the Centre for the Study of Evolution, University of Sussex, Brighton BN1 9QG, UK. E-mail: a.c.eyre-walker@sussex.ac.uks

Bazin *et al*. conducted their analysis by first compiling an impressive DNA diversity data set for both nuclear and mitochondrial DNA. Using an automated system, they searched the GenBank and EMBL databases for instances in which the same gene had been sequenced in multiple individuals of a species. This yielded, after some restrictions to improve data quality, 417 species for which they had diversity data for nuclear DNA and 1683 species for mitochondrial DNA. They also analyzed a data set of 912 species for which allozyme diversity data were available.

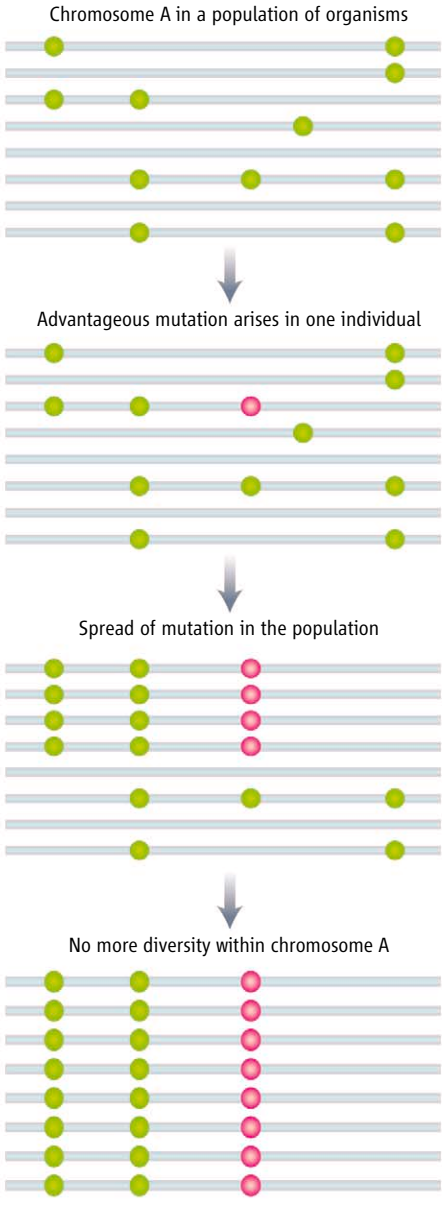
Unfortunately, the census population size is not known for the vast majority of organisms, so Bazin and colleagues used a number of phylogenetic and ecological factors to test whether population size and diversity were correlated.

For example, they tested whether invertebrates had higher diversities than vertebrates, and whether (within each group) marine organisms had higher diversities than terrestrial or freshwater organisms. In all comparisons, nuclear genes followed the expected pattern—the group that was expected to have the bigger population size had higher diversity. However, this pattern was not observed for mitochondrial DNA—there was remarkably little difference in diversity between any of the groups.

Why do nuclear DNA and mitochondrial DNA behave so differently? There are a number of possibilities, but the most conspicuous difference between the two genomes is the lack, or very low level, of recombination in mitochondrial DNA. Bazin *et al*. suggest that because of this low level of recombination, mitochondrial DNA might be particularly prone to a process called genetic hitchhiking (see the figure). When an advantageous mutation spreads through a population, it reduces the genetic variation at loci that are linked to it. This is most easily seen if we consider the case in which a single advantageous mutation arises in a chromosome when there is no recombination. Once the advantageous mutation has spread through the population, all individuals share the same copy of that chromosome and there is no diversity within the chromosome until new mutations arise.

Genetic hitchhiking does not by itself explain why genetic diversity in mitochondrial DNA is independent of population size. To complete the explanation, we must further assume that the rate of adaptive evolution is correlated to population size. There are two reasons why this might be the case. First, more adaptive mutations will occur in big populations because there are more individuals to mutate. Second, selection is more effective, so even adaptive mutations that are very weakly selected may become fixed in big populations. If this is the case, then we might have the following situation: As the population size increases, genetic diversity will tend to increase. But at the same time, the number of adaptive substitutions (and hence genetic hitchhiking events) increases, thus reducing the level of genetic diversity. Gillespie (*2*) has shown that these two forces tend to cancel each other out, leaving the genetic diversity largely independent of population size. In contrast, for nuclear DNA, recombination reduces the effects of genetic hitchhiking, and diversity increases with population size.

In support of their hypothesis, Bazin *et al*. show that the neutrality index, a measurement of selection, is significantly lower in invertebrate than in vertebrate mitochondrial DNA, and that the median values are less than 1 in both groups. A neutrality index of less than 1 indicates that adaptive amino acid substitutions have occurred, and one can calculate the proportion of amino acid substitutions that were adaptive from the neutrality index. Using the median values of the neutrality index given by Bazin *et al*., it is possible to estimate that 58% of amino acid substitutions are adaptive in invertebrate mitochondrial DNA and 12% in vertebrates. Given that the ratio of the nonsynonymous to synonymous amino acid substitution rate is also higher in invertebrates, this suggests that the rate of adaptive substitution, relative to the mutation rate, is higher in invertebrate mitochondrial DNA.




**Genetic hitchhiking.** A new advantageous mutation (red dot) arises in an individual on a chromosome that also contains other genetic variations (green dots). As the advantageous mutation spreads through members of the population, it reduces the genetic diversity until there is no diversity left. Mitochondrial DNA may be prone to this process.

Interestingly, humans are an exception to the pattern seen by Bazin *et al*. If the authors are correct, then the effective population size estimated from mitochondrial DNA should be lower than that estimated from autosomal DNA. This is not what we see in humans; the effective population sizes estimated from autosomal DNA, Y-chromosome DNA, and mitochondrial DNA are all approximately 10,000 (*3*). Does this mean that Bazin *et al*. are incorrect? Probably not. It may be that humans have such small effective population sizes that adaptive evolution in the mitochondrial genome is very rare; the neutrality index in human mitochondrial DNA, and perhaps nuclear DNA, certainly gives no indication of adaptive evolution. Although nuclear diversity follows the expected pattern, with more diversity in organisms that are expected to have bigger population sizes, the differences are remarkably small; synonymous diversity varies by less than a factor of 10, and allozyme diversity by less than a factor of 4. This is striking given that the population sizes of marsupials and mussels, for example, must differ by many orders of magnitude, and one would expect diversity to be linearly related to population size. This observation is not new for allozyme data (*4*), but it is the first time this pattern has been so clearly illustrated for synonymous diversity in nuclear genes. The lack of a strong correlation between diversity and population size in nuclear DNA may also reflect the effects of genetic hitchhiking.

If Bazin *et al*. are correct and the diversity of mitochondrial DNA, and perhaps nuclear DNA, is limited by repeated adaptive substitution, then the rate of adaptation is dependent on the rate of mutation, not the rate at which the environment changes. This does not mean that environmental changes are not important; it simply means that no species is ever perfectly adapted and that adaptive mutations can always occur in any species. This further implies that most adaptive mutations arise de novo and do not come from genetic variation that is already segregating in the population.

The fact that the diversity of mitochondrial DNA does not appear to reflect population size adds further weight to the arguments of others (*5*) that mitochondrial DNA may be of only limited utility in understanding ecological, genetic, and evolutionary processes. It is ironic that the lack of recombination, once seen as a great asset of mitochondrial DNA, may be something of a problem in this context.

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ASTRONOMY

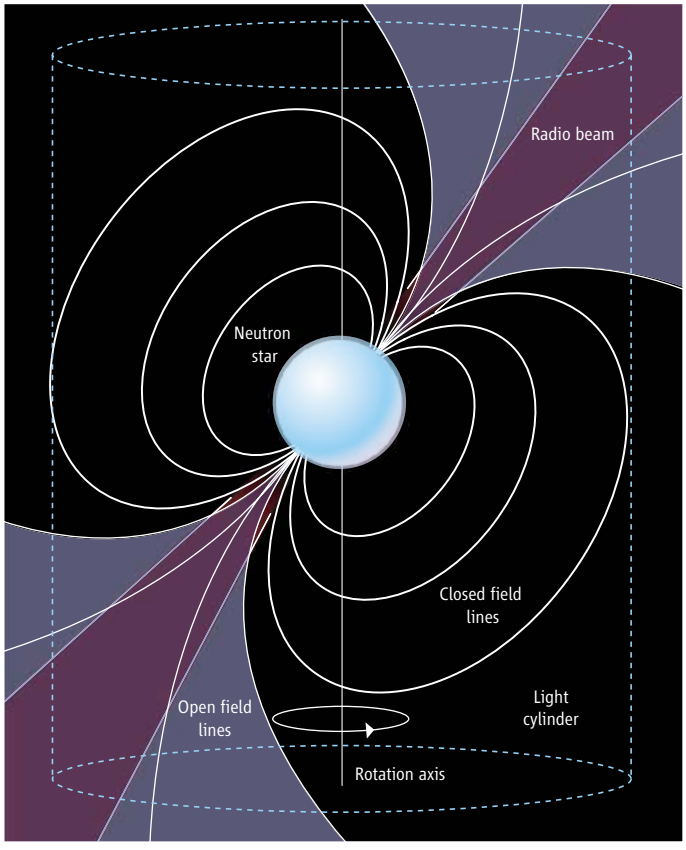
# Pulsar Magnetospheres and Pulsar Death

Rotating neutron stars, or pulsars, slow down with time. New observations of an unusual intermittent pulsar provide direct evidence that this slowing is partially caused by outflow of plasma.

**E. P. J. van den Heuvel**

Pulsars are rapidly spinning neutron stars whose lighthouse-like beams of radio waves sweep Earth, producing highly regular pulses with periods typically of order 1 s. Their spin periods gradually increase with time, and it was realized in 1968 that this must be due to the braking effect of a magnetic field some $10^{12}$ times stronger than that of Earth (*1*). The rotation of the magnetic field causes the emission of magnetic dipole radiation, which exerts a torque that slows down the neutron star's rotation (*2*, *3*). It was suggested in 1969 that an additional braking torque is exerted by the outflow of the relativistic particles that produce the pulsed radio emission of the pulsar (*4*). Now, after 37 years and the discovery of over 1500 pulsars, clear evidence for the existence and strength of this "pulsar wind" torque has finally been found in the behavior of a highly peculiar pulsar, as described by Kramer *et al.* (*5*) on page 549 of this issue. This is an important breakthrough, because it gives quantitative information on the strengths of the magnetospheric electric currents that generate the pulsar's pulsed radiation.

Neutron stars and black holes are the most dense and compact objects known in nature, and they have the strongest gravitational fields. They are formed by the collapse of the burned-out core of a massive star, accompanied by a supernova explosion in which the envelope of the star is violently ejected. With a mass some 400,000 times that of Earth and a diameter of 20 km (smaller than Los Angeles), a neutron star is essentially a giant atomic nucleus held together by gravity. The gravitational attraction at its surface is about 11 orders of magnitude greater than on the surface of Earth. Calculations of the structure of neutron stars show that although their interior consists, in large part, of neutrons, these stars have a solid crust of a few kilometers thickness that consists of a lattice of atomic nuclei and electrons. This crust has a very high electrical conductivity that anchors the magnetic field by allowing very strong electric currents to flow in the crust. The field is expected to be mainly dipolar—like that of Earth and Jupiter—and as in these planets, it has an axis that is inclined with respect to the rotation axis (see the figure).




**Putting on the brakes.** The rotating magnetic field of a pulsar creates a very strong electric field that pulls charged particles out of the solid crust of the star. These particles emit gamma rays, which, in turn, interact with the magnetic field, setting in motion an avalanche of electron-positron pairs. Close to the neutron star's polar caps, the flow of pair particles creates magnetospheric electric currents that produce the observed beams of radio waves (red). The currents and the pulsar wind of plasma (blue) flowing out from the magnetosphere along the open field lines that cross the light cylinder exert a torque on the magnetic field lines that slows down the neutron star's rotation. This braking torque was predicted 37 years ago, and now, direct observational evidence for its existence has been found (*5*).

The existence of a pulsar wind of relativistic particles flowing out into space was put forward by Goldreich and Julian (*4*). They calculated the strength of the electric field generated by the rotating magnetic field and found that on certain areas of the neutron star surface, this field creates a force on particles that is far stronger than the gravitational force that holds them down. Therefore, electrons can escape from these parts of the star surface and are accelerated by the electric field along the magnetic field lines, achieving a highly relativistic speed after only a few centimeters of travel. From other parts of the neutron star surface, positively charged particles (nuclei) can be drawn out, such that the magnetosphere of the neutron star is filled with an outflowing ionized plasma. As shown in the figure, this plasma can flow out into space only along open magnetic field lines, which originate in the neutron star's polar caps around its magnetic poles, and cross the so-called light-cylinder, where the field lines would rotate with the velocity of light..

In two seminal papers (*6*, *7*), Sturrock argued that the magnetospheric plasma must mainly consist of electrons and their antiparticles, positrons. An accelerated electric charge spontaneously emits electromagnetic radiation, and, owing to the very high energy of the electrons, this radiation will be high-energy gamma rays. (Indeed, several young pulsars have been observed to emit pulsed gamma radiation.) In a magnetic field, high-energy gamma-ray photons are spontaneously converted into electron-positron pairs by the process of pair creation. The resulting electron and positron are again accelerated in the electric field and, after a few centimeters, again produce gamma photons that produce pairs, and so on. Thus, by pulling just one electron out of the neutron star surface, a cascade of electron-positron pairs is created, which fills the magnetosphere of the neutron star with a pair plasma. (The density of this plasma is so low that an electron sel-

The author is with the Anton Pannekoek Astronomical Institute and the Center for High Energy Astrophysics, University of Amsterdam, Kruislaan 403, 1098SJ Amsterdam, Netherlands and Kavli Institute for Theoretical Physics, University of California Santa Barbara, Santa Barbara, CA 93106-4030, USA. E-mail: edvdh@science.uva.nl

dom again meets a positron to be annihilated back into gamma-ray photons.)

Sturrock (*7*) argued that the electric field accelerates the electrons and positrons in opposite directions (the positrons toward the neutron star), such that these particles form sheets of electrical current. Plasma oscillations caused by periodic instabilities in these sheets are expected to produce the pulsar's radio emission. That young supernova remnants, such as the Crab Nebula, are filled with a plasma of relativistic electrons was known since the mid-1950s, when it was discovered that the blue light from this nebula is synchrotron radiation, produced by highly relativistic electrons moving in an ordered magnetic field in the nebula (*8*).

We have known since 1968 that the pulsar wind of relativistic particles that is seen in a handful of young supernova remnants is produced by their central young pulsars. For the vast majority of the ordinary older radio pulsars that no longer have such detectable pulsar wind nebulae, there were no observational clues to whether a pulsar wind exists and plays any role in slowing down the neutron star's rotation.

Now Kramer *et al*. (*5*) find that the pulsar PSR B1931+24, which is an apparently ordinary pulsar with a period of 0.813 s, exhibits a remarkable behavior not seen in any other pulsar before: It periodically switches off completely for periods of about 25 to 35 days, and then switches on again for active phases lasting 5 to 10 days. Most surprisingly, it appears that during the on phases, its rotation slows down about twice as fast as during the off phases. Because the pulsar radio beams are generated by the acceleration of relativistic charged particles in the magnetosphere of the neutron star, the complete absence of radio emission during the off phases indicates that the current of charged particles flowing out from the polar caps is shut down completely during these phases. In this case, the pulsar is slowed down only by the emission of (vacuum) magnetic dipole radiation. The difference in spindown rate of a factor of two then indicates that about half of the spindown rate of this pulsar in the on phase is due to the pulsar wind (it is also possible that when the wind plasma is present, the magnetic dipole radiation is short circuited completely and the slowdown is entirely produced by the pulsar wind). Because this pulsar has a pulse period and surface dipole magnetic field strength ($4.5 \times 10^{12}$ G) in the range observed for ordinary radio pulsars, this remarkable result suggests that in ordinary radio pulsars, the pulsar wind plays an essential role in slowing down the rotation of the neutron star.

Apart from this discovery giving us a direct glimpse of the currents that produce the pulsar's radio radiation, it also may shed new light on many other aspects of the physics and evolution of these stars. For example, we may learn from this pulsar much more about the "death" of radio pulsars. All pulsars spin down, and no radio pulsars with periods longer than about 8 s are known, thus it is clear that neutron stars stop functioning as radio pulsars when their spin period exceeds a certain maximum value. On average, this point is reached when the pulsar is about 10 million years old. This death is ascribed to the fact that when the neutron star's rotation slows down, the strength of the electric field generated by its rotating magnetic field decreases. At some point, the magnetic field becomes so weak that either it can no longer pull charged particles out of the neutron star surface (*4*) or, if it still can, the electrons no longer are accelerated to sufficient energy to produce gamma ray photons of sufficient energy (> 0.5 MeV) to initiate pair creation (*7*). In either case, the pulsar wind is shut off and the pulsed radio emission stops. PSR B1931+24 seems close to reaching this point, as it is already off about 70% of the time. This behavior is also interesting because this pulsar gives us, for the first time, information about what happens to the pulsar spindown after the pulsar has turned off and has disappeared into the pulsar "graveyard" (which, in our galaxy, is expected to already contain about one billion dead pulsars). It shows that once in the graveyard, a pulsar keeps spinning down due to magnetic dipole radiation, although at about half the pace at which it was spinning down while it was still active. This means that in the graveyard, pulsars may reach very long spin periods. For example, a pulsar with a magnetic field of $10^{13}$ G will reach a spin period of over 80 s in one billion years. This is just one example of the interesting implications of this discovery, and many more should follow now that we have gained direct information about the electric currents flowing in pulsar magnetospheres.

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NEUROSCIENCE

# A Neuronal Receptor for Botulinum Toxin

**Reinhard Jahn**

Muscle paralysis induced by botulinum toxin A is used cosmetically to eliminate wrinkles. It preferentially enters activated neurons by binding to a synaptic vesicle protein that becomes exposed after transmitter release.

Some say poison, some say "wonder drug." Either way, the neurotoxins produced by the bacterium *Clostridium botulinum* have gained notoriety or fame as powerful inhibitors of neuronal synaptic transmission. Their effects range from preventing wrinkled skin to causing severe food poisoning and respiratory failure. Botulinum neurotoxins bind to the surface of nerve terminals, gain access to the cytoplasm, and block the release of neurotransmitters into the synapse. Although the mechanism by which these toxins enter neurons was worked out more than a decade ago, the receptors responsible for toxin binding and internalization have only partially been known. On page 592 of this issue, Dong *et al*. (*1*) identify SV2, a conserved membrane protein of synaptic vesicles, as the receptor for botulinum neurotoxin A.

Protein toxins are highly sophisticated weapons that bacteria use to manipulate or kill eukaryotic cells or even entire organisms with minimal effort, thus providing a source of nutrients for their survival and proliferation. Many toxins contain two polypeptide chains, referred to as A and B chains, which have distinct roles. The B chain binds to the surface of the target cell, commandeers the endocytotic pathway to facilitate internalization of the toxin, and then mediates translocation of the A chain into the cytoplasm. The A chain is an enzyme that executes the damage by modifying selected cellular target proteins or altering the amounts of intracellular signaling molecules. Consequently, signaling pathways are derailed, resulting in cell malfunction or cell death (*2*).

The seven botulinum neurotoxins and the

The author is in the Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, 37077 Göttingen, Germany. E-mail: rjahn@gwdg.de

related tetanus toxin are the most potent of all bacterial protein toxins. Botulism can be contracted by ingesting spoiled food, whereas tetanus toxin must enter the bloodstream to become effective. Once they are in the circulation, the toxins bind with very high specificity to the surface of peripheral nerve terminals, where they are taken up by endocytosis. Botulinum neurotoxins act locally and block neurotransmitter release, leading to muscular paralysis. Tetanus toxin is transported along the axons into the spinal cord where it crosses the synaptic cleft and inhibits the nerve terminals of glycinergic interneurons. As a result, inhibitory feedback regulation is lost, leading to overexcitation of the neuron and muscle cramps (*3*, *4*). When small doses of botulinum neurotoxins (particularly type A) are injected into muscle, a locally confined and long-lasting neuromuscular block develops. This is because acetylcholine, the neurotransmitter that triggers muscle contractions, cannot be released. This effect has given rise to the widespread use of these toxins to relieve muscle spasms and to cosmetically "remove" wrinkles by local muscle paralysis.

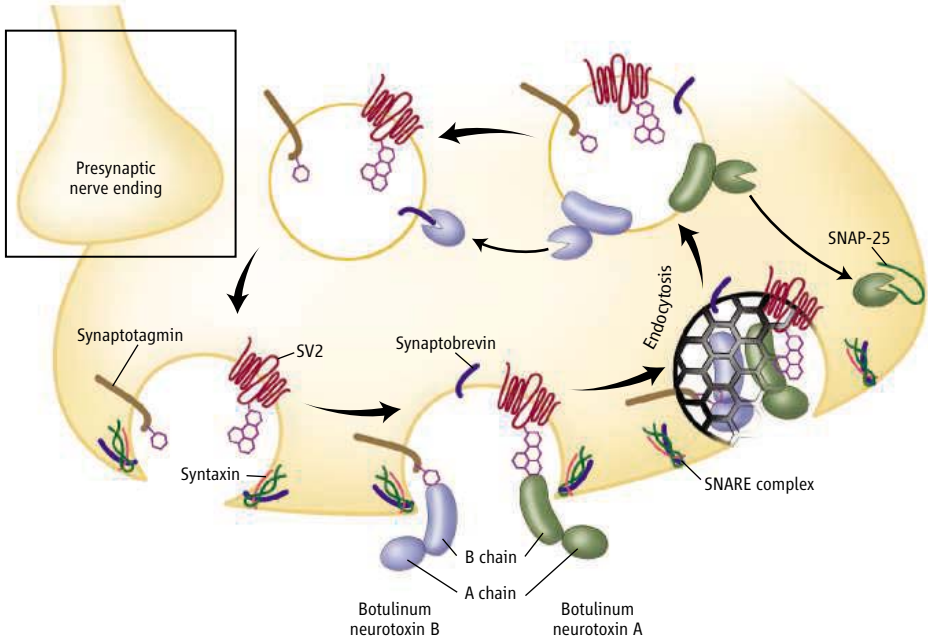
The mechanisms by which these neurotoxins block transmission are well understood (*4*). The A chains function as zinc-dependent endoproteases that selectively cleave synaptic SNARE (SNAP receptor) proteins. SNAREs are small, conserved membrane proteins that include synaptobrevin on synaptic vesicles and SNAP-25 and syntaxin on the plasma membrane. Each A chain is specialized for only one of these proteins. SNAREs mediate the fusion of synaptic vesicles with the presynaptic membrane, so when any of the SNARE proteins in presynaptic nerve terminals are cleaved by an A chain, transmitter release is inhibited. In contrast, the receptors for the B chains have been harder to identify, although the binding affinity of the toxins to the presynaptic membrane is very high. These difficulties arise probably because two independent low- to medium-affinity receptors need to cooperate for efficient toxin binding. One of these receptors has been known for many years: It consists of gangliosides, complex glycolipids that are specifically localized on the outer surface of neuronal plasma membranes. The second receptor was suspected to be a protein, but its identity was unknown.

Twelve years ago, Nishiki and colleagues (*5*) identified the synaptic vesicle protein synaptotagmin as a coreceptor for botulinum neurotoxin B. Synaptotagmin is a calcium sensor that couples calcium influx into the neuron to the fast phase of neurotransmitter release (*6*). For the toxin, however, other features of synaptotagmin are beneficial: It is a transmembrane glycoprotein and thus, when the synaptic vesicle fuses with the plasma membrane during exocytosis, synaptotagmin is exposed on the surface of the nerve terminal. Subsequently, synaptotagmin is efficiently internalized, giving bound toxin a free ride into the terminal. After endocytosis, the vesicles acidify, which triggers translocation of the A chain into the cytoplasm (see the figure). However, other botulinum neurotoxins do not use synaptotagmin as receptor, such as the clinically widely used botulinum toxin A.

Dong *et al*. (*1*) now identify SV2 as the receptor for botulinum neurotoxin A (see the figure). SV2 is a conserved membrane protein of synaptic vesicles that is structurally related to neurotransmitter transporters. In the mammalian brain, there are three closely related isoforms of SV2 (*7*), and they all function as toxin receptors. Toxin binding was assigned to a large, highly glycosylated loop that is conserved between the isoforms. Neurons obtained from mice lacking two of the three SV2 isoforms are unable to bind and internalize the toxin, but binding is restored when SV2 is expressed in these neurons. Furthermore, these mice are less sensitive to toxin poisoning. Thus, a second membrane protein of synaptic vesicles has now been identified as the receptor for a botulinum neurotoxin.

SV2 was proposed to be involved in presynaptic calcium regulation, but the molecular mechanism underlying this function is not known (*6*). For the toxin, the function of SV2 does not matter. As one of the few synaptic vesicle proteins with large luminal domains, it provides a convenient anchor for internalization just as synaptotagmin does for botulinum neurotoxin B. Exposure of these receptors at the surface of nerve terminals also increases when a synapse releases more acetylcholine, thus giving the toxins an additional advantage of preferably attacking active nerve terminals.

The identification of two structurally unrelated receptors for botulinum neurotoxins A and B sheds new light on a remarkable evolutionary adaptation. Although the toxins originated from a common ancestral precursor protein, both the A and the B chains have evolved to interact with structurally very different proteins. Botulinum neurotoxin A binds to SV2 and cleaves the SNARE protein SNAP-25, whereas botulinum neurotoxin B binds to synaptotagmin and cleaves the SNARE protein synaptobrevin. What is conserved, however, is the purpose of the toxins: to block neuromuscular synaptic transmission and to kill the cell or organism that then serves as fermenter for bacterial proliferation.




**Point of entry.** Botulinum neurotoxins A and B bind to the SV2 and synaptotagmin, respectively, and are internalized by endocytosis into vesicles coated with clathrin (black and gray cage-like structure). Endocytotic vesicles acidify, which triggers membrane insertion of the B chains and translocation of the A chains across the membrane into the cytoplasm. The A chain of botulinum neurotoxin A cleaves SNAP-25, whereas the A chain of neurotoxin B cleaves synaptobrevin. Cleavage of either SNARE protein interferes with the formation of stable SNARE complexes that are required for exocytotic fusion of synaptic vesicles with the plasma membrane.

ILLUSTRATION: C. CAIN/*SCIENCE*

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INTERNATIONAL

# AAAS, Partners Launch Global Web Site on Sustainability Science

With interest rising worldwide in sustainability science and related social issues, AAAS's Center for Science, Innovation, and Sustainability has launched a new Web site to serve as the hub of an international network in the challenging years ahead.

The site (http://sustainabilityscience.org) is an advanced, one-stop shop for scholars, governments, agencies, and others working to understand how humanity can grow and develop in an environmentally sustainable way. It offers a virtual library, discussion forums, commentary, and international listings of events and programs on sustainability. And it does so with a no-frills visual approach that is accessible even to users with limited bandwidth.

"The forum brings together leading scientists from developed and developing countries to begin grappling with some of the most fundamental science questions at the nexus of environment and development," said Vaughan Turekian, AAAS chief international officer.

"Forum: Science and Innovation for Sustainable Development" is an updated and reinvigorated version of a site hosted for several years at Harvard University. The concept grew out of discussions at the path-breaking Friibergh Workshop on Sustainability Science, which drew 24 influential scientists and scholars to Sweden in 2000. They and others had come to believe that biology, chemistry, hydrology, and other individual fields of science, operating independently, had limited ability to chart the dynamic interactions between nature and society. A new, interdisciplinary approach was needed.

The Friibergh workshop gave rise to the Initiative on Science and Technology for Sustainability (ISTS). The Web site originally was seen as a depot for Friibergh documents and deliberations; soon, however, the organizing committee began to envision a bigger role.

The old site—like sustainability science itself—"has outgrown its original construct," said geographer Robert Kates, a 1991 U.S. Medal of Science winner and co-convenor of the initiative. At first, sustainability was defined largely in scientific terms, he explained in an e-mail interview. But after a series of meetings around the globe, it was clear that social issues and issues related to the interaction of society and nature are just as important.

"The new Forum will give equal prominence to these using at a global level the United Nations' Millennium Development Goals and the water, energy, health, agriculture, biodiversity, and urban sectoral framework of the United Nations," he said.

The effort to bring the Web site to AAAS was led by Shere Abbott, then the association's chief international officer; Abbott and Lars Bromley, a senior program associate in the AAAS Office of International Initiatives, collaborated on the project with ISTS leaders, including Kates and William Clark, an ecologist and key initiative organizer based at Harvard.

Currently, the forum has more than 300 members in 41 countries. That number is expected to jump as word of the Web site circulates and outreach activities restart.

"What I especially value about the commitment of AAAS to the forum is its experience and concern for the next generation of science and technology, both in its Web sites and in its creative programs of introducing young scientists to policy," Kates said. "Sustainability science measures a transition to sustainability in terms of generations. Its task to support such a transition will extend across this century and many of its most productive contributors are still unborn."

SCIENCE AND SOCIETY

# Ayalas' Passion for Knowledge Shines at AAAS Event

Francisco J. Ayala is a trailblazer in evolutionary genetics and an outspoken defender of scientific integrity in public school classrooms. Hana Ayala is pursuing a visionary plan to make tourism, conservation, and science a self-sustaining engine for economic development. They are the archetype of a scientific power couple, traveling the globe to advance their ideas.

But their varied interests are based on a shared belief: Knowledge should be used to benefit humanity. And their passion for knowledge was vivid when they took the stage in the packed AAAS auditorium, 23 March in Washington, D.C., for a 90-minute conversation about their ideas, their work, and their strategies for maintaining marital harmony even with a breakneck travel schedule.

Czech-born Hana Ayala calls her concept Pangea World, after the super-continent that broke apart to form the continents we know today. Thus far, she has focused largely on Panama and Fiji, developing a model to aid local economies by creating financial incentives for conservation and encouraging scientific research. When the plan is realized, travelers will be able to lodge in what she calls IQ Resorts, immersed in exotic settings and a sophisticated scientific milieu.

The partnership between tourism and science may represent "the greatest untapped reserve for funding an exposure to science," she said, "the greatest untapped resource for revolutionizing the business success and quality of the hotel industry, and thirdly, it is also the greatest untapped means of elevating conservation to a powerful economic force."

Francisco J. Ayala (left), AAAS CEO Alan I. Leshner, and Hana Ayala in a salon-style conversation at AAAS.

Francisco Ayala, born in Madrid, is a former Roman Catholic priest and now University Professor in biology, philosophy, and logic at the University of California, Irvine. His work has led to dramatic advances in the prevention and treatment of diseases such as malaria. He served as AAAS president in 1995. In 2001, he won the U.S. National Medal of Science.

"Most mainstream theologians, and most people who have read the Bible thoughtfully, realize that the Bible, it is not an elementary book of biology, or an elementary book of cosmology or of physics," he told the audience. "It is a travesty to interpret the Bible that way."

For more information on the Ayalas and to view the video of their evening at AAAS, see www.aaas.org/webextras/.

# Reefs of the Deep: The Biology and Geology of Cold-Water Coral Ecosystems

J. Murray Roberts,[1] Andrew J. Wheeler,[2] André Freiwald[3]

Coral reefs are generally associated with shallow tropical seas; however, recent deep-ocean exploration using advanced acoustics and submersibles has revealed unexpectedly widespread and diverse coral ecosystems in deep waters on continental shelves, slopes, seamounts, and ridge systems around the world. Advances reviewed here include the use of corals as paleoclimatic archives and their biogeological functioning, biodiversity, and biogeography. Threats to these fragile, long-lived, and rich ecosystems are mounting: The impacts of deep-water trawling are already widespread, and effects of ocean acidification are potentially devastating.

Cold-water corals have been known since the 18th century. Only recently, as fishery and oil exploration activities in deeper waters have increased, have developments in acoustic survey techniques and access to submersibles revealed the scale and abundance of cold-water coral ecosystems (*1*, *2*). Corals occur individually, as isolated colonies, in small patch reefs several meters across, or they form large reefs and giant carbonate mounds up to 300 m high and several kilometers in diameter over many thousands to millions of years. Because of their age and growth rates, reefs contain high-resolution records of long-term climate change and may also be important speciation centers in the deep sea. Recent research suggests a coupling between the reef fauna and surface productivity, with reef development controlled by the interplay of local hydrography and sedimentary dynamics.

## Corals, Reefs, and Carbonate Mounds

Cold-water corals are cnidarians encompassing stony corals (Scleractinia), soft corals (Octocorallia, including "precious" corals, gorgonian sea fans, and bamboo corals), black corals (Antipatharia), and hydrocorals (Stylasteridae) (Fig.1). They are azooxanthellate (i.e., lacking symbiotic dinoflagellates) and often form colonies supported by a common skeleton, providing structural habitat for other species. Here we focus on scleractinian reef framework–forming species. Gorgonian and antipatharian corals, although not reef-forming, can develop dense assemblages that form important structural habitats (Fig. 1). These are mostly outside the scope of this review, although their value as paleoclimatic archives is discussed below.

Species, habitats, and ecosystems discussed here are distinct from those of tropical coral reefs and are specifically associated with colder conditions, often in deep offshore waters (*3*). Reefs and mounds tend to cluster in "provinces," where specific hydrodynamic and food supply conditions favor coral growth. Some provinces are characterized by small mound features; e.g., the Darwin Mounds in the northern Rockall Trough (*4*, *5*), or giant carbonate mounds where reefs have become repeatedly established since the Late Pliocene/Pleistocene; e.g., in the Porcupine Seabight, NE Atlantic (*6*, *7*).

## Reef Distribution, Genesis, and Development

Cold-water corals are largely restricted to oceanic waters and temperatures between 4° and 12°C. These conditions are generally found in relatively shallow waters (~50 to 1000 m) at high latitudes, and at great depths (up to 4000 m) beneath warm water masses at low latitudes. Approximately 800 species of reef-building scleractinians are described in shallow waters, yet fewer than 10 are known to make substantial deep-water reef frameworks (*1*, *8*). Of these, we have an incomplete view of their global distribution (Fig. 2), which remains skewed by the geographically varied levels of research activity and the bias of deep-water mapping initiatives to the developed world. Despite this, some intriguing patterns in their global biogeography are becoming evident. Cold-water scleractinian species diversity is highest around the Philippines, with global distribution influ-

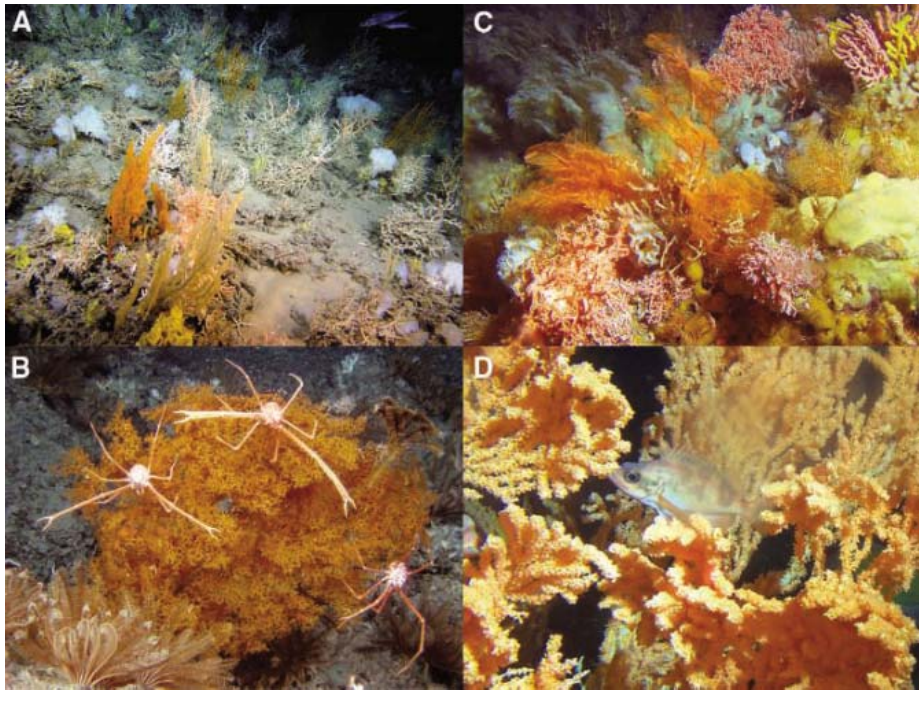


**Fig. 1.** Cold-water coral reef fauna. (**A**) Sloping flank of the Galway carbonate mound colonized by scleractinian and gorgonian corals and glass sponges. (**B**) Currently undescribed antipatharian coral (*Leiopathes* sp., pers. comm. D. Opresko) and associated anomuran crustaceans from the Twin Mounds, Porcupine Bank (NE Atlantic). [Images courtesy Alfred-Wegener-Institut für Polar und Meeresforschung and Institut Français de Recherche pour l'Exploitation de la Mer] (**C**) Diverse coral and sponge fauna recently discovered off the Aleutian Islands. [Image courtesy of A. Lindner, National Oceanic and Atmospheric Administration (NOAA) Fisheries] (**D**) Sharpchin rockfish (*Sebastes* sp.) among gorgonian corals (*Primnoa* sp.) in the Gulf of Alaska (N Pacific). [Image courtesy of V. O'Connell, Alaska Department of Fish and Game]

[1]Scottish Association for Marine Science, Dunstaffnage Marine Laboratory, Oban, Argyll, PA37 1QA, UK. [2]Department of Geology and Environmental Research Institute, Donovan's Road, University College Cork, Cork, Ireland. [3]Institute of Paleontology, Erlangen University, Loewenichstrasse 28, D-91054, Erlangen, Germany.

enced by seawater carbonate chemistry (*9*). Recent work shows a striking relationship between the number of cold-water scleractinian occurrences and the depth of the aragonite saturation horizon (ASH). This may help explain the abundance of coral records in the NE Atlantic, where the ASH is >2000 m deep, compared with the paucity of records from the N Pacific, where the ASH is as shallow as 50 to 600 m and the coral fauna is dominated by octocorals and stylasterids (*9*, *10*).

Reefs develop after an initial settlement of a coral larva to a hard substratum. As a coral grows, polyps in older portions die, and the skeleton becomes increasingly vulnerable to bioeroders (notably, clionid sponges) and mechanical breakage. Bioeroded skeletons may break, fall to the seabed, and extend the perimeter of the reef patch. These processes are fundamental in creating the reef framework that, over time, baffles and traps mobile sediment. Provided that coral growth keeps pace with sediment infill, localized mound formation is initiated (*11*, *12*). The development of deep-water reef mounds and their colonization can be thought of in a cyclical sense, with the associated community predicted to vary with the stage of reef development and available microhabitats (Fig. 3).

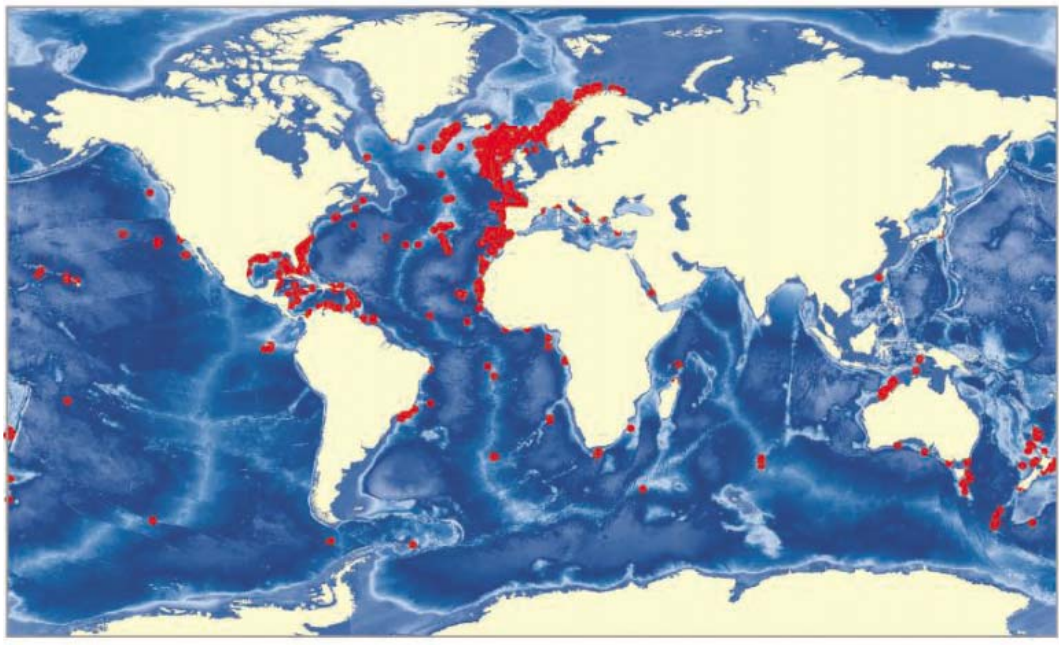

**Fig. 2.** Current global distribution of reef framework–forming cold-water corals [modified from (*1*)].

*Lophelia pertusa* reefs on the Norwegian shelf and carbonate mounds in the Porcupine Seabight were thought to be related to light hydrocarbon seepage, a concept later developed into a hydraulic theory of cold-water coral reef development (*13*). However, isotopic compositions of coral skeleton and tissue are not compatible with a seepage-based food chain (*14*), and although some reefs are reported close to seeps and pockmarks, many, if not most, are not. In May 2005, the International Ocean Drilling Program (IODP) Expedition 307 drilled to the base of the Challenger Mound in the Porcupine Seabight and found no gas accumulation beneath or within the mound and no evidence that mound growth was initiated by hydrocarbon seepage (*7*). Conversely, mound growth appears to be initiated at several sites over a wide area upon an unconformity followed by a rapid period of mound growth and coalescence. We believe this may indicate that clusters of small mounds may, over time and under favorable environmental conditions, form giant carbonate mounds.

### Trophic Dynamics

Unlike the predictions of the hydraulic theory, recent research shows that cold-water corals are fueled by primary productivity in surface waters and subsequent food transport to the sea floor (*14*). Corals are frequently reported from sites with locally accelerated currents, or from areas of the continental slope where internal tidal waves enhance seabed food supply. Around topographic highs, such as the Porcupine Bank in the NE Atlantic, currents trap nutrient-rich waters above the bank that, under certain conditions of bottom slope and density stratification, may drain slowly off-slope through the benthic boundary layer (Ekman drainage) and supply food to the coral reefs associated with large carbonate mounds on its slopes (*15*). Recent studies in the Darwin Mounds found fresh labile material, including lipids and polyunsaturated fatty acids, present at depths of almost 1 km (*16*). Coral lipid and nitrogen isotope compositional analysis suggests that *L. pertusa* and *Madrepora oculata* rely predominantly on a zooplankton diet; but compared with *M. oculata*, *L. pertusa* is enriched in monounsaturated fatty acids and $\delta^{15}N$, has far larger polyps, and has been seen capturing live zooplankton in the field. It is therefore possible that these two species adopt different feeding strategies, although species-specific metabolic differences cannot be ruled out (*17*). Recent work on shallow-water scleractinians reinforces the view that corals are cosmopolitan consumers able to feed from suspended sediments, dissolved organic matter, bacteria, protozoans, and both zoo- and phytoplankton (*18*). Detrital and resuspended materials are likely to be important food sources for corals in deep waters. Sediment-trap studies show that phytodetritus, fecal pellets, and zooplankton were the most substantial sources of particulate carbon available to cold-water coral communities on Galicia Bank in the NE Atlantic (*14*). Although currently unquantified, diurnally migrating zooplankton and over-wintering populations of calanoid copepods are likely to be ecologically important prey items.

### Reproductive Ecology and Population Genetics

Little is known about the reproduction of cold-water corals, despite its fundamental importance. Most shallow-water scleractinians are hermaphrodites, but the majority of cold-water corals studied to date are gonochoristic (have separate sexes). Seasonally enhanced food flux associated with spring phytoplankton blooms greatly influences benthic carbon flux and reproductive periodicity in deep-sea fauna. In the NE Atlantic, it seems that gamete production in *L. pertusa* follows phytodetrital food fall, and this species is likely to spawn before the following spring (*19*).

The application of molecular tools to cold-water coral populations is a powerful approach to elucidate taxonomic and systematic relationships. For example, ribosomal RNA (rRNA) sequencing suggests that *M. oculata* may have been historically misclassified (*20*). The degree of reef connectivity can also be examined by using a molecular approach. Microsatellite and ribosomal internal transcribed spacer (ITS) sequence analyses indicate that *L. pertusa* is not a panmictic population in the NE Atlantic but seems to form discrete fjord and shelf populations (*21*). In the Darwin Mounds, microsatellite analysis shows that the coral population is clonal (*21*), and a histological study found no reproductive corals (*22*). In the Pacific Ocean, microsatellite studies suggest that the precious gorgonian coral *Corallium*

*lauuense* is suffering inbreeding depression on Hawaiian seamounts, perhaps because of fishing pressure for its skeleton, which is used in jewelry making (*23*). Such information is vital to develop conservation policies, and we anticipate major advances in this area in the coming years as more genetic markers are developed and applied.

### Biodiversity and Endemism

Cold-water corals are arguably the most three-dimensionally complex habitats in the deep ocean, providing niches for many species. For example, we know that over 1300 species have been found living on *L. pertusa* reefs in the NE Atlantic (*24*). Their biodiversity may be comparable to that found on tropical coral reefs, but few quantitative studies allowing regional comparisons have been made. We understand little of the functional relationships between species on cold-water coral reefs, and the reefs' importance as a fish habitat is unclear (*8*).

We also understand little of the connectivity between reef provinces. Seamounts trap ocean currents producing localized circulation patterns. Under these conditions, larvae could be retained, which would limit species' dispersal, promote local adaptation, and potentially enhance rates of speciation (*25*). Although very few of the estimated 30,000 to 50,000 seamounts have been studied, species endemism appears to be high. For example, up to 34% of species on SW Pacific seamounts were newly discovered and potentially endemic. Because there were few common species between seamounts in this region, it is possible that, especially along ridge systems, seamounts may be analogous to island groups (*26*). Cold-water coral reefs are frequently reported from seamounts; therefore, given their species diversity, propensity to localized circulation patterns, and longevity, cold-water coral reefs may be major speciation centers.

### Longevity and Paleoclimatic Archives

Cold-water coral reef and mound development in the NE Atlantic reflect environmental change over geological time scales corresponding to recurrent glacial cycles. In northern Europe, Scandinavian reefs date from the Holocene after the retreat of the Pleistocene ice sheet ~10,000 to 14,000 years ago. Stratigraphic studies from the giant carbonate mounds off Ireland show pronounced depositional cycles of coral-rich and hemipelagic sedimentation associated with glacial-interglacial periods extending back to at least the early Pleistocene, albeit with substantial hiatuses (*7*, *12*). In the Mediterranean Sea, off NW Africa, and on the mid-Atlantic ridge beyond the southern limit of the ice sheets, U/Th dating suggests continuous cold-water coral growth over the last 50,000 years (*27*). Given that reef framework–forming corals such as *L. pertusa* cannot survive in water masses <4°C but can rapidly colonize new anthropogenic substrata (*28*), it seems likely that high-latitude reefs have repeatedly diminished during glacial periods and flourished during interglacial periods. This would limit their potential role as speciation centers. Conversely, we suggest that coral ecosystems at lower latitudes, especially those on seamounts where biodiversity and endemism are known to be high, are likely to be important speciation centers and glacial refugia in the deep sea.

Given their longevity over geological time scales, cosmopolitan distributions, and banded skeletal structure, cold-water corals are important paleo-environmental archives. There are two broad categories of studies: (i) those that estimate past seawater temperatures from skeletal chemistry and (ii) those that follow the ventilation history of the ocean by dating skeletal material. Both are fundamental in reconstructing climate history.

Stable oxygen isotopes from biogenic carbonates have been used for many years to estimate paleo-seawater temperatures and salinities. However, scleractinian coral skeletons do not form in isotopic equilibrium with seawater, and in azooxanthellate corals, stable isotopes of oxygen and carbon are strongly correlated. These kinetic effects may relate to the rate of coral calcification that is probably driven by seasonal factors: notably, annual phytodetrital deposition (*29*). Despite this, stable isotopes from cold-water corals have been used to derive seawater temperatures (*30*). Interpreting chronologies from scleractinian framework-forming species like *L. pertusa* is complicated by cryptic banding patterns and complex skeletal morphologies, even though they are widespread and well-preserved in the fossil record. Recent work on *Enallopsammia rostrata* shows that even though the banding pattern is not annual, it may be possible to interpret annual chronology using $^{210}$Pb as a time proxy (*31*). There is now good evidence that the tree-like stems of cold-water octocorals are annually banded (*32*), unlike *E. rostrata*, and Mg/Ca ratio analysis of gorgonian and bamboo corals has yielded convincing paleo-temperatures (*33*, *34*).

Shifts in deep-ocean circulation patterns profoundly affect global climate, and there is now

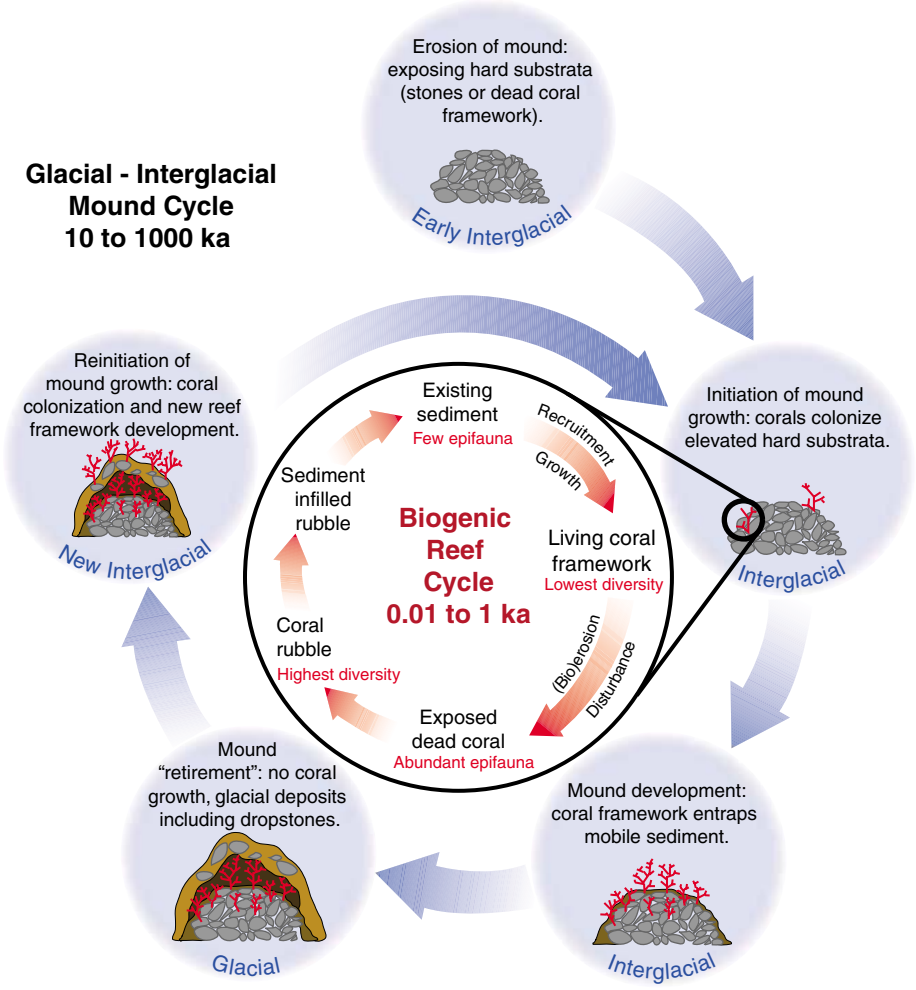


**Fig. 3.** Schematic illustration showing the following: (**Outer circle**) Cyclic stages of carbonate mound growth from initiation, development, retirement, and recolonization. (**Inner circle**) Smaller scale cycle of reef microhabitats, succession, and faunal diversity.

evidence that cold-water corals have recorded these oceanic shifts in their skeletons. Because U/Th dating yields coral age and $^{14}C$ dating provides coral age plus the age of the inorganic carbon in seawater, complementary U/Th and $^{14}C$ studies infer the age of the seawater in which the coral grew (*35*). Adkins *et al*. (*36*) suggest that the solitary coral *Desmophyllum cristagalli* experienced shoaling of "young," low-nutrient North Atlantic deep water and replacement with "old," high-nutrient southern source water. Shoaling of more than 200 m occurred very rapidly, i.e., during the coral's 160-year lifetime, and coincided with the transition to the Bølling-Alleröd warming period. This approach has now been used to study ventilation histories in the Southern Ocean (*37*) and North Atlantic (*38*).

## Threats

Although we are only now starting to realize the ubiquity of cold-water coral reefs, their biodiversity, and value as paleoclimatic resources, human activities threaten these ecosystems in three ways: (i) bottom trawling causes damage, (ii) hydrocarbon drilling and seabed mining have potential impacts, and (iii) ocean acidification has potentially severe effects on calcifying reef fauna.

There is global evidence that these habitats have been damaged by trawling for deep-water fish, causing severe physical damage from which recovery to former reef status will take several hundreds or thousands of years, if at all (Fig. 4) (*1*, *39*–*41*). Several nations, including Canada, Norway, UK, and USA, have responded by closing cold-water coral habitats to bottom fishing. Beyond territorial waters on the High Seas, no individual nation has jurisdiction, and any conservation measures would need to be developed using the United Nations Convention on the Law of the Sea (*1*).

Compared with widespread evidence for physical damage to reef structures from bottom trawling, there is little evidence that hydrocarbon exploitation substantially threatens cold-water coral ecosystems. *L. pertusa* colonizes North Sea oil platforms and seems to have formed a self-seeding population, despite proximity to drilling discharges (*42*). Greatest concern is over the potential for drill cuttings to smother reef fauna (*43*), but such effects would be highly localized when compared with the extent of seabed affected by bottom trawling. To date, there has been little interest in mining the rich mineral deposits found in some seamounts and along oceanic ridge systems. However, mining activities risk causing local extinctions on seamounts supporting endemic species.

Perhaps the most insidious threat to cold-water coral reef ecosystems is from ocean acidification. There is general consensus that atmospheric carbon dioxide levels are rising sharply, and modeled scenarios suggest that this could cause the greatest increase in ocean acidification over the last 300 million years (*44*). Current research predicts that tropical coral calcification would be reduced by up to 54% if atmospheric carbon dioxide doubled (*45*). There have been no studies to examine these effects on cold-water corals, but given the lowered carbonate saturation state at higher latitudes and deeper waters, these species may be even more vulnerable. In addition to the effect acidification could have on coral calcification, modeling studies predict that the depth at which aragonite dissolves could shallow by several hundred meters, thereby raising the prospect that areas once suitable for cold-water coral growth will become inhospitable (*10*, *45*, *46*).

## Conclusions

This Review highlights recent major advances in our understanding of cold-water coral ecosystems after intensified research efforts across the biological and geological sciences. Of particular importance are studies into paleoclimatic archives, biodiversity, and habitat destruction. Emerging concepts are the importance of cold-water coral reefs as speciation centers and deep-water glacial refugia, major threats posed by the impacts of ocean acidification, and evidence confirming hydrodynamic biogeological carbonate mound growth processes. We expect to see continued advances in many areas, particularly reproductive and genetic studies, biodiversity, biogeography, species interaction, and ecological studies where time series of data are required. Sea floor observatories are anticipated to play an important future role (*47*).

Despite the inherent difficulties of studying these habitats, there is an urgent need for sound information on which to base long-term management plans. Bottom trawling, often on the High Seas, has damaged cold-water coral ecosystems, and we do not understand the long-term implications of ocean acidification. The vast majority of cold-water coral reef studies so far are from the developed world; therefore, a need exists to transfer expertise in deep-water surveys to the developing world and to begin unified ocean basin–scale comparisons if we are to appreciate the global connectivity and importance of these reefs of the deep.

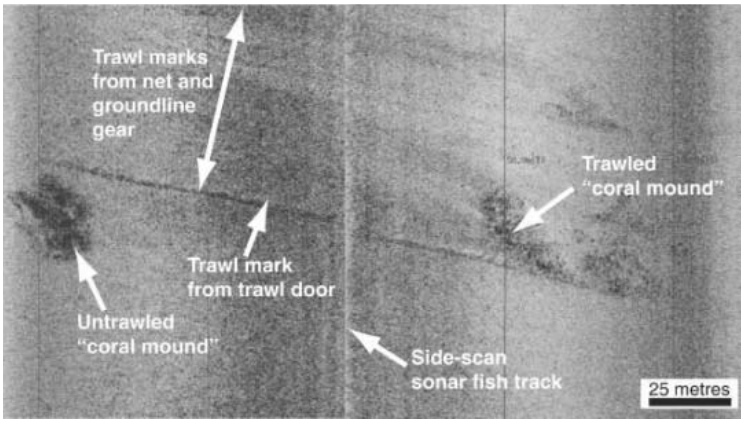


**Fig. 4.** Side-scan sonograph showing trawl damage to the Darwin Mounds at almost 1-km water depth in the Rockall Trough (NE Atlantic). Reduced backscatter (lighter tones) from the trawl-damaged mound strongly suggests decreased coral abundance [modified from (*5*)].

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48. We thank the following for permission to reproduce images: Alfred-Wegener-Institut für Polar und Meeresforschung, Institut Français de Recherche pour l'Exploitation de la Mer, United Nations Environment Programme-World Conservation Monitoring Centre, R. P. Stone, and A. Lindner. We thank L-A. Henry, D. S. Schoeman, R. Leakey, J. A. Howe, and B. Dorschel for helpful comments on the manuscript and A. J. Davies and M. Kozachenko for help with illustrations. This work was supported in part by the HERMES project, European Commission contract no. GOCE-CT-2005-511234, funded by the European Commission's Sixth Framework Programme under the priority Sustainable Development, Global Change, and Ecosystems.


## Supporting Online Material

www.sciencemag.org/cgi/content/full/312/5773/543/DC1
SOM Text
References

10.1126/science.1119861

# Santorini Eruption Radiocarbon Dated to 1627–1600 B.C.

Walter L. Friedrich,[1]* Bernd Kromer,[2] Michael Friedrich,[2,3] Jan Heinemeier,[4] Tom Pfeiffer,[1] Sahra Talamo[2]

The Minoan eruption of Santorini (Thera) in Greece spread a huge fan of volcanic ash deposits over the Eastern Mediterranean region. Worldwide effects have been ascribed to the eruption: sulphuric acid and fine ash particles in the Greenland Ice Sheet, climatic disturbances in China, and frost damage to trees in Ireland and California. On Santorini, the eruption produced a thick layer of pumice and ash (fig. S1) (*1*) that buried flourishing Bronze Age settlements. Left behind is Akrotiri, a "Pompeii of the ancient Aegean," which is currently being excavated (*2*). Precise dating of this eruption is important, because the tephra layer acts as a universal time marker of Late Bronze Age contexts in the Eastern Mediterranean region.

On the basis of linkages between the Aegean and Egyptian cultures (*3*), some archaeologists have traditionally placed the Minoan eruption around the mid- to late-16th century B.C., whereas evidence from ice cores, tree rings, and a large number of radiocarbon dates favor a date 100 years earlier (*4*). Radiocarbon dates on short-lived organic material, like seeds, from Akrotiri on Santorini have been of limited precision because of a plateau in the radiocarbon calibration curve for part of this time trajectory. Even by applying refined statistical analysis on the most suitable series of radiocarbon dated samples grouped by archaeological evidence, it has not yet been possible to date the eruption of Santorini more precisely than ranging (2σ) from 1663 to 1599 B.C. (*5*).

We have found (*6*) a branch from an olive tree that was buried alive in tephra on Santorini, with branches of the crown partly preserved in life position (Fig. 1A and fig. S1) (*1*). The horizontal position of the seven molds of branches in the pumice 1 to 3 m above its base and remnants of olive leaves and twigs covered by the pumice further support our claim that the olive was buried alive. We obtained a series of radiocarbon dates from a defined sequence of tree rings in the branch that can be wiggle-matched to the latest radiocarbon calibration curve IntCal04 (Fig. 1B). One problem with olive trees is that they form irregular, barely visible rings. We thus used x-ray tomography to identify 72 rings in a section of the branch with preserved bark (Fig. 1C) (*1*). We divided the section into four consecutive groups of rings and obtained $^{14}C$ dates for each (*1*). Using the calibration program OxCal (*7*), we determined the calibrated age range of the outermost ring to 1621–1605 B.C. (1σ, 68% confidence) or 1627–1600 B.C. (2σ, 95% confidence). Even when we take into account an uncertainty of 50% in the ring count, potentially caused by growth irregularities of olive, these limits are increased by only a decade (table S2). We also excluded a significant local offset of the $^{14}C$ ages by volcanic $CO_2$, because then it would be impossible to match our $^{14}C$ sequence anywhere to the shape of the calibration curve (Fig. 1C).

Our wiggle-matched sequence adds to the already strong evidence of an eruption date in the late 17th century B.C. It is the first accurately (close to annually) defined sequence based on an object buried alive by the eruption. A date around 1520 B.C. or later, as assumed by some archaeologists working with Egyptian contexts, is not consistent, even within 3σ (99.7% confidence), with our result, which consequently suggests a flaw in either their linkage of the Aegean to the Egyptian chronology or in the chronology itself for the relevant time range.

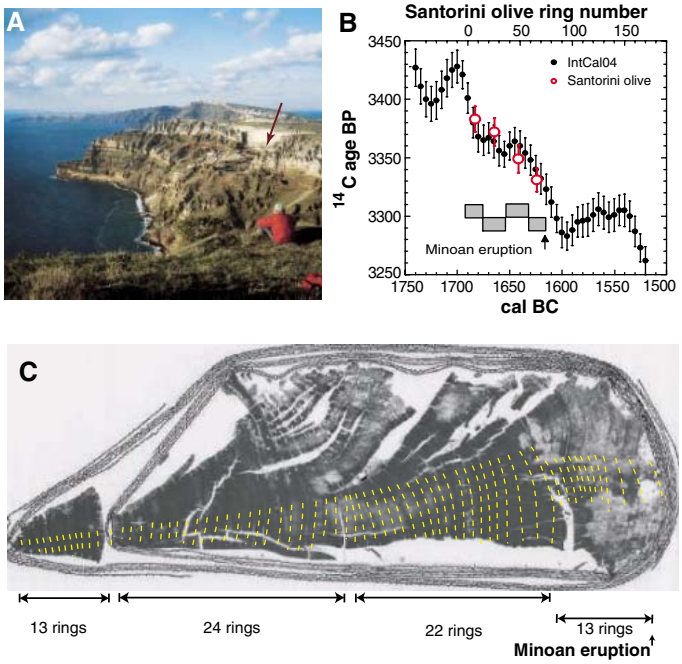


**Fig. 1.** (**A**) The arrow marks the site where an olive tree was found buried under the <60-m-thick pumice layer of the Minoan eruption on top of the caldera wall on Thera. (**B**) $^{14}C$ dates of the four segments of the olive section wiggle-matched to the calibration curve IntCal04. The relative position of the segments in the branch is indicated by the bars. BP, years before the present. Error bars indicate 1 SD. (**C**) X-ray tomography of a section of the olive branch, with yellow lines indicating identified growth rings. The four dated segments are indicated by arrows.


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8. We thank H. Jannopoulou-Akyla and G. Nomikos for providing recent olive material for comparison; I. Pfeifer-Schäller for providing the x-ray tomography imagery; S. Manning, A. McBirney, R. Wilson, and H. Loft Nielsen for comments; and G. Nielsen and M. Dybdahl for technical assistance.


## Supporting Online Material

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Table S1
References

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[1]Department of Earth Sciences, University of Aarhus, DK-8000 Aarhus, C.F. Moellers Allé 1120, Denmark. [2]Heidelberger Akademie der Wissenschaften, Institut fur Umweltphysik, Im Neuenheimer Feld 229, D-69120 Heidelberg, Germany. [3]Institute of Botany, Hohenheim University, D-70593 Stuttgart, Germany. [4]Accelerator Mass Spectrometry $^{14}C$ Dating Centre, Department of Physics and Astronomy, University of Aarhus, DK-8000 Aarhus C, Denmark.

*To whom correspondence should be addressed. E-mail: walter@geo.au.dk

# A Periodically Active Pulsar Giving Insight into Magnetospheric Physics

M. Kramer, A. G. Lyne, J. T. O'Brien, C. A. Jordan, D. R. Lorimer

PSR B1931+24 (J1933+2421) behaves as an ordinary isolated radio pulsar during active phases that are 5 to 10 days long. However, when the radio emission ceases, it switches off in less than 10 seconds and remains undetectable for the next 25 to 35 days, then switches on again. This pattern repeats quasi-periodically. The origin of this behavior is unclear. Even more remarkably, the pulsar rotation slows down 50% faster when it is on than when it is off. This indicates a massive increase in magnetospheric currents when the pulsar switches on, proving that pulsar wind plays a substantial role in pulsar spin-down. This allows us, for the first time, to estimate the magnetospheric currents in a pulsar magnetosphere during the occurrence of radio emission.

Pulsar radio emission is generally understood in terms of beams of coherent plasma radiation from highly relativistic particles above the magnetic pole of a rotating neutron star, producing pulses as the beam crosses Earth. However, there is no satisfactory theory that explains the radio emission or even the magnetospheric conditions that determine whether a neutron star emits at radio wavelengths. Observationally, the typical active lifetime of a radio pulsar is estimated to be about 10 million years, during which, on long time scales, pulsar emission is essentially steady (*1*). It was therefore surprising when a unique activity pattern was found for the pulsar PSR B1931+24 (also known as J1933+2421) during routine pulsar timing observations with the 76-m Lovell Telescope at the Jodrell Bank Observatory in the United Kingdom.

The pulsar had been considered to be a seemingly ordinary pulsar, with a spin period of 813 ms (*2*) and a typical rotational frequency derivative of $\dot{\nu} = -12.2 \times 10^{-15}$ Hz s$^{-1}$ (Table 1) (*3*). It was noted that it exhibits considerable short-term rotational instabilities intrinsic to the pulsar, known as timing noise, but shows no evidence indicating the presence of any stellar companion. It became clear a few years ago that the pulsar was not detected in many of the regular observations and that the flux density distribution was bimodal, the pulsar being either on or off. Figure 1 shows the best-sampled data span, which covers a 20-month period between 1999 and 2001 and demonstrates the quasi-periodic pattern of the on/off sequences. The power spectrum of the data reveals a strong ~35-day periodicity with two further harmonics, which reflect the duty cycle of the switching pattern (Fig. 1). Studying a much longer time series from 1998 to 2005, including some intervals of less densely sampled data, we find that the periodicities are persistent but slowly vary with time over a period ranging from 30 to 40 days. No other known pulsar behaves this way.

To investigate the nature of the variations, we examined the rotation rate of the pulsar over a 160-day period (Fig. 2A). The variation is dominated by a decrease in rotational frequency, which is typical for pulsars. However, inspection of the longer sequences of the available data on the on phases in the diagram reveals that the rate of decrease is even more rapid during these phases, indicating values of the rotational frequency first derivative that are higher than the average value. This suggests a simple model in which the frequency derivative has different values during the off and on phases. Such a model accurately describes the short-term timing variations that are seen relative to a simple long-term slowdown model (Fig. 2B). Over the 160-day period shown, the pulsar was monitored almost daily, so that the switching times were well defined, and a model could be fitted to the data with good precision. The addition of a single extra parameter (that is, two values of the frequency derivative rather than one) reduced the timing residuals by a factor of 20 and provides an entirely satisfactory description of the data. A similar fitting procedure was applied to other well-sampled sections of the data and produced consistent model parameters (Table 1), giving values for the rotational frequency derivatives of $\dot{\nu}_{\rm off} = -10.8(2) \times 10^{-15}$ Hz s$^{-1}$ and $\dot{\nu}_{\rm on} = -16.3(4) \times 10^{-15}$ Hz s$^{-1}$. These values indicate that there is an ~50% increase in spin-down rate of the neutron star when the pulsar is on.

The observed quasi-periodicity in pulsar activity and its time scale have never been seen before as a pulsar emission phenomenon and are accompanied by massive changes in the rotational slowdown rate. This raises a number of questions. Why does the emission switch on and off? Why is the activity quasi-periodic? Why is the pulsar spinning down faster when it is on?

On the shortest, pulse-to-pulse time scales, intrinsic flux density variations are often observed in pulsar radio emission. The most extreme case is displayed by a small group of pulsars that are known to exhibit "nulls" in their emission; that is, the random onset of a sudden obvious lack of pulsar emission, typically lasting between one and a few dozen pulsar rotation periods (*4*). An acceptable explanation for such nulling, which appears to be the complete failure of the radiation mechanism, is still missing. This nulling previously represented the longest known time scales for an intrinsic disappearance of pulsar emission. The facts that the off periods of PSR B1931+24 are five orders of magnitude

**Table 1.** Observed and derived parameters of PSR B1931+24. Standard (1σ) errors are given in parentheses after the values and are in units of the least significant digit. The distance is estimated from the dispersion measure and a model for the interstellar free electron distribution (*14*). Definitions for characteristic age, surface magnetic field, and spin-down luminosity can be found in (*5*).

| Parameter | Value |
|---|---|
| Right ascension (J2000) | $19^h33^m37^s.832(14)$ |
| Declination (J2000) | +24°36′39″.6(4) |
| Epoch of frequency (modified Julian day) | 50629.0 |
| Rotational frequency ν (Hz) | 1.2289688061(1) |
| Rotational frequency derivative $\dot{\nu}$ (Hz s$^{-1}$) | $-12.2488(10) \times 10^{-15}$ |
| Rotational frequency derivative on $\dot{\nu}_{\rm on}$ (Hz s$^{-1}$) | $-16.3(4) \times 10^{-15}$ |
| Rotational frequency derivative off $\dot{\nu}_{\rm off}$ (Hz s$^{-1}$) | $-10.8(2) \times 10^{-15}$ |
| Dispersion measure DM (cm$^{-3}$ pc) | 106.03(6) |
| Flux density during on phases at 1390 MHz (μJy) | 1000(300) |
| Flux density during off phases at 1390 MHz (μJy) | ≤2 |
| Flux density during on phases at 430 MHz (μJy) | 7500(1500) |
| Flux density during off phases at 430 MHz (μJy) | ≤40 |
| Active duty cycle (%) | 19(5) |
| Characteristic age τ (million years) | 1.6 |
| Surface magnetic field strength $B$ (T) | $2.6 \times 10^8$ |
| Spin-down luminosity $\dot{E}$ (W) | $5.9 \times 10^{25}$ |
| Distance (kpc) | ~4.6 |

Jodrell Bank Observatory, University of Manchester, Macclesfield, SK11 9DL, UK.

longer than typical nulling periods, that the activity pattern is quasi-periodic, and that not a single null has been observed during on periods strongly suggest that the phenomenon found here is different from nulling.

The approximate 35-day period might be attributed to free precession, although we find no evidence of expected (*5*) profile changes. Although switches between states are rare events, we have been able to observe one switch from on to off that occurred within 10 s, the time resolution being limited by the signal-to-noise ratio of the observations. The sudden change and the quasi-periodicity point toward a relaxation oscillation of unknown nature within the pulsar system, rather than precession.

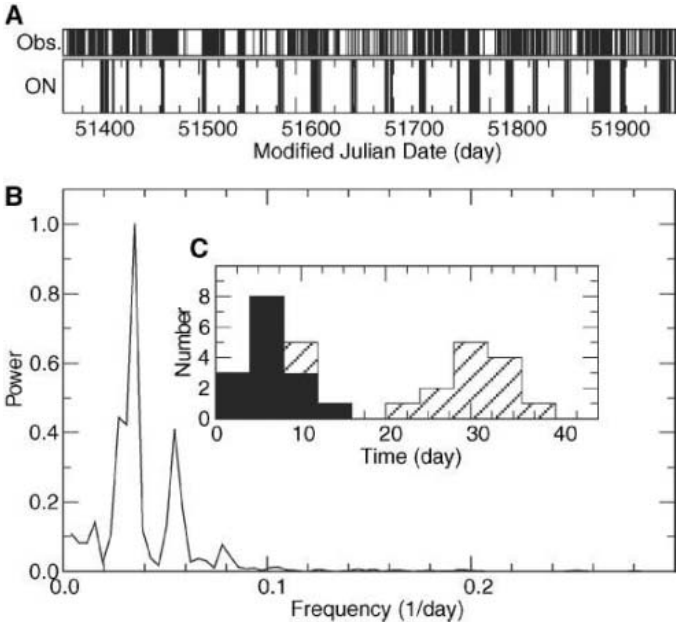


**Fig. 1.** Time variation of the radio emission of PSR B1931+24. During the on phases, the pulsar is easy to detect and has the stable long-term intrinsic flux density associated with most normal pulsars. Since 1998, the pulsar has been observed as frequently as twice a day. (**A**) A typical sequence of observations covering a 20-month interval is indicated by the black lines. It shows, respectively, the times of observation and the times when PSR B1931+24 was on. It is clear that the pulsar is not visible for ~80% of the time. (**B**) The appearance of the pulsar is quasi-periodic in nature, demonstrated by the power spectrum of the intensity obtained from the Fourier transform of the autocorrelation function of the mean pulse flux density obtained over the same 20-month interval. (**C**) Histograms of the durations of the on (solid) and off (hatched) phases. In off phases, integration over several weeks shows that any pulsed signal has a mean flux density of less than than 2 μJy at 1400 MHz (1 Jy = $10^{-26}$ W m$^{-2}$ Hz$^{-1}$). A deep observation with the Arecibo telescope on Puerto Rico provides an upper flux density limit of 40 μJy at 430 MHz. Simultaneous observations at frequencies between 430 and 1400 MHz show that the presence or absence of radio emission is a broadband phenomenon.

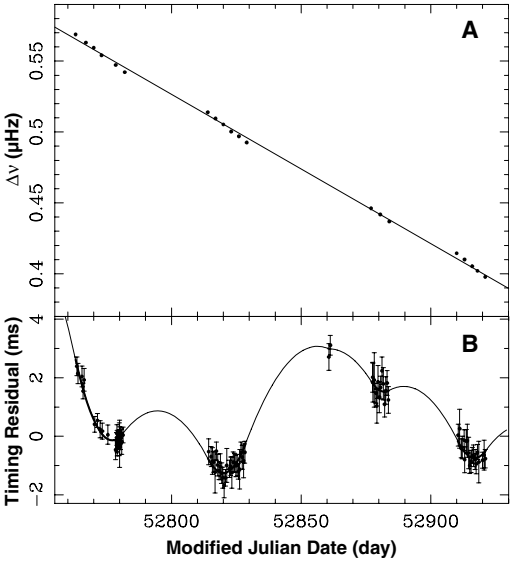


**Fig. 2.** Variation of the rotational frequency of PSR B1931+24. (**A**) Evolution of the rotational frequency over a 160-day period. The errors in the measurement of the data points are smaller than the size of the symbols. The variation is dominated by the long-term spin-down: a gradual decrease in rotational frequency. The best-fit straight line through the points is shown, representing a frequency derivative of $\dot{\nu} = -12.2 \times 10^{-15}$ Hz s$^{-1}$. However, an inspection of the data reveals that, when the pulsar is on, the slope and hence the magnitude of the derivative are even greater. This suggests a model in which the frequency derivative has different values during the off and on periods. (**B**) Examining this possibility more closely, timing residuals, which are the differences between the observed pulse arrival times and those derived from a simple long-term slowdown model (Table 1), show substantial short-term variations. Over the period covered by this figure, the pulsar was monitored almost daily, so that the off and on periods were well defined and the model described above could be fitted to the data with good precision. The fitted model is shown as a continuous line overlying the data points and clearly describes the data very well.

What can cause the radio emission to cut off so quickly? The energy associated with the radio emission from pulsars accounts for only a very small fraction of the pulsar's slowdown energy, which may suggest that the disappearance of radiation is simply due to the failure of the coherence condition in the emission process (*6*). However, in that case, the long time scales of millions of pulsar rotations are hard to understand. One alternative explanation is that there is a more global failure of charged particle currents in the magnetosphere.

The large changes in slowdown rate that accompany the changes in radio emission can also be explained by the presence or absence of a plasma whose current flow provides an additional braking torque on the neutron star. In this model, the open field lines above the magnetic pole become depleted of charged radiating particles during the off phases when the rotational slowdown, $\dot{\nu}_{off}$, is caused by a torque dominated by magnetic dipole radiation (*7*, *8*). When the pulsar is on, the decrease in rotational frequency, $\dot{\nu}_{on}$, is enhanced by an additional torque provided by the outflowing plasma, $T \sim \frac{2}{3c} I_{pc} B_0 R_{pc}^2$, where $B_0$ is the dipole magnetic field at the neutron star surface and $I_{pc} \sim \pi R_{pc}^2 \rho c$ is the electric current along the field lines crossing the polar cap, having radius of $R_{pc}$ (*9*). [In order to be consistent with existing literature such as (*9*), we quote formulas in centimeter-gram-second units but refer to numerical values in SI units.] The charge density of the current can be estimated from the difference in loss in rotational energy during the on and off phases. When the pulsar is on, the observed energy loss, $\dot{E}_{on} = 4\pi^2 I \nu \dot{\nu}_{on}$, is the result of the sum of the magnetic dipole braking as seen during the off phases, $\dot{E}_{off} = 4\pi^2 I \nu \dot{\nu}_{off}$, and the energy loss caused by the outflowing current, $\dot{E}_{wind} = 2\pi T \nu$; that is, $\dot{E}_{on} = \dot{E}_{off} + \dot{E}_{wind}$, where $I$ is the moment of inertia of the neutron star. From the difference in spin-down rates between off and on phases, $\Delta\dot{\nu} = \dot{\nu}_{off} - \dot{\nu}_{on}$, we can therefore calculate the charge density $\rho = 3I\Delta\dot{\nu}/R_{pc}^4 B_0$ by computing the magnetic field $B_0 = 3.2 \times 10^{15} \sqrt{-\dot{\nu}_{OFF}/\nu^3}$ Tesla and the polar cap radius $R_P = \sqrt{2\pi R^3 \nu / c}$ for a neutron star with radius $R$ = 10 km and a moment of inertia of $I = 10^{38}$ kg m$^2$ (*10*). We find that the plasma current that is associated with radio emission carries a charge density of $\rho = 0.034$ C m$^{-3}$. This is remarkably close to the charge density $\rho_{GJ} = B_0 \nu / c$ in the Goldreich-Julian model of a pulsar magnetosphere (*11*); that is, $\rho_{GJ} = 0.033$ C m$^{-3}$.

Such charge density is sufficient to explain the change in the neutron star torque, but it is not clear what determines the long time scales or what could be responsible for suddenly changing the plasma flow in the magnetosphere. In that respect, understanding the cessation of radiation

that we see in PSR B1931+24 may ultimately also help us to understand ordinary nulling. Whatever the cause is, it is conceivable that the onset of pulsar emission may be a violent event that may be revealed by high-energy observations. Although an archival search for x-ray or γ-ray counterparts for PSR B1931+24 has not been successful, the relatively large distance from the pulsar (~4.6 kpc) and arbitrary viewing periods may make such a detection unlikely.

The relation between the presence of pulsar emission via radiating particles and the increased spin-down rate of the neutron star provides strong evidence that a pulsar wind plays a substantial role in the pulsar braking mechanism. Although this has been suggested in the past (*12*), direct observational evidence has been missing so far. As a consequence of the wind's contribution to the pulsar spin-down, magnetic fields estimated for normal pulsars from their observed spin-down rates are likely to be overestimated.

The discovery of PSR B1931+24's behavior suggests that many more such objects exist in the Galaxy but have been overlooked so far because they were not active during either the search or confirmation observations. The periodic transient source serendipitously found recently in the direction of the galactic center (*13*) may turn out to be a short–time-scale version of PSR B1931+24 and hence to be a radio pulsar. In general, the time scales involved in the observed activity patterns of these sources pose challenges for observations scheduled with current telescopes. Instead, future telescopes with multibeaming capabilities, like the Square-Kilometre-Array or the Low Frequency Array, which will provide continuous monitoring of such sources, are needed to probe such time scales, which are still almost completely unexplored in most areas of astronomy.

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# Quantum-Dot Spin-State Preparation with Near-Unity Fidelity


**Mete Atatüre,[1]* Jan Dreiser,[1] Antonio Badolato,[1] Alexander Högele,[1,2] Khaled Karrai,[2] Atac Imamoglu[1]***



**We have demonstrated laser cooling of a single electron spin trapped in a semiconductor quantum dot. Optical coupling of electronic spin states was achieved using resonant excitation of the charged quantum dot (trion) transitions along with the heavy-light hole mixing, which leads to weak yet finite rates for spin-flip Raman scattering. With this mechanism, the electron spin can be cooled from 4.2 to 0.020 kelvin, as confirmed by the strength of the induced Pauli blockade of the trion absorption. Within the framework of quantum information processing, this corresponds to a spin-state preparation with a fidelity exceeding 99.8%.**


Semiconductor quantum dots (QDs) have been referred to as artificial atoms because of their discrete atom-like states. Photoluminescence (PL) studies of single QDs under nonresonant excitation have led to the generation of single photons (*1*, *2*) and cavity-quantum electrodynamics in the weak-coupling (*2*–*4*) and strong-coupling (*5*–*7*) regimes: all indicators of a quantum optical system. Similarly, resonant excitation has enabled the observation of Rabi oscillations (*8*) and coherent manipulation of excitons (*9*). These advances, in turn, have strengthened various proposals, including those regarding optical accessing of spins in QDs (*10*). However, from the perspective of quantum information processing (*11*), the ability to prepare, manipulate, and detect a spin qubit optically in solid-state systems is yet to be demonstrated.

We have demonstrated the high-fidelity preparation of a QD spin state via laser cooling [optical pumping (*12*)]. Using the Pauli blockade strength of the corresponding optical transitions as a means to infer the electron spin state, we showed that spin cooling due to spontaneous spin-flip Raman scattering can dominate over the heating introduced by hyperfine-induced spin-flip or cotunneling events. This allowed us to cool the spin temperature of an electron from 4.2 K (determined by the heat bath) down to 20 mK. By controlling the relative strength of these processes via gate voltage and magnetic field, we can tune the system from the regime of an isolated artificial atom to that of a quantum-confined solid-state system coupled either to a charge or a spin reservoir.

The experiments were performed on molecular-beam-epitaxy–grown single self-assembled InAs/GaAs QDs in a gated heterostructure, where the only difference as compared to the one used in (*13*) was the 35-nm tunnel barrier between the QD layer and the electron reservoir. In similar devices, a gate voltage applied between the ohmic and the Schottky contacts provides deterministic charging of QDs with signatures in the optical transitions (*14*). We performed the initial characterization of our QDs by conventional micrometer-resolution photoluminescence (μ-PL) spectroscopy at 4.2 K to determine the voltage range for each charging state, along with the associated optical transition frequencies. Figure 1A shows a typical gate sweep for our device, and the labels $X^0$ and $X^{1-}$ identify the relevant optical transitions for our experiments: those from an empty QD and those from a single-electron–charged QD. We then carried out magneto-optical spectroscopy of the $X^{1-}$ transition to extract the excitonic Zeeman splitting of 30 GHz/T. Having characterized the basic optical properties of the QD, we switched to resonant excitation using differential transmission technique: Fig. 1B shows a typical absorption plot at 0 T as a single-frequency laser is tuned across the trion transition. The details of this technique (*15*) along with its advantages in spin-selective measurements (*16*) can be found in previous works.

A single-electron–charged QD in the trion picture is analogous to the four-level system illustrated in Fig. 2A, where state $|\uparrow\downarrow, \blacktriangledown\rangle$ ($|\uparrow\downarrow, \blacktriangle\rangle$) corresponds to the QD with two ground-state electrons forming a singlet and a ground-state hole with angular momentum projection $J_z = -3/2$ (3/2) along the growth direction. The strong trion transitions, $|\uparrow\downarrow, \blacktriangledown\rangle \leftrightarrow |\downarrow\rangle$ and $|\uparrow\downarrow, \blacktriangle\rangle \leftrightarrow |\uparrow\rangle$, leave the resident electron spin unaltered, whereas the weak transitions, $|\uparrow\downarrow, \blacktriangledown\rangle \leftrightarrow |\uparrow\rangle$ and $|\uparrow\downarrow, \blacktriangle\rangle \leftrightarrow |\downarrow\rangle$, lead to a net spin-flip of the resident electron. The latter transitions are ideally forbidden by the optical selection rules; nevertheless, inherent heavy-light hole mixing


[1]Institute of Quantum Electronics, ETH Zurich, CH-8093 Zurich, Switzerland. [2]Center for NanoScience and Sektion Physik, Ludwig-Maximilians-Universität, 80539 Munich, Germany.

*To whom correspondence should be addressed. E-mail: atature@phys.ethz.ch (M.A.); imamoglu@phys.ethz.ch (A.I.)

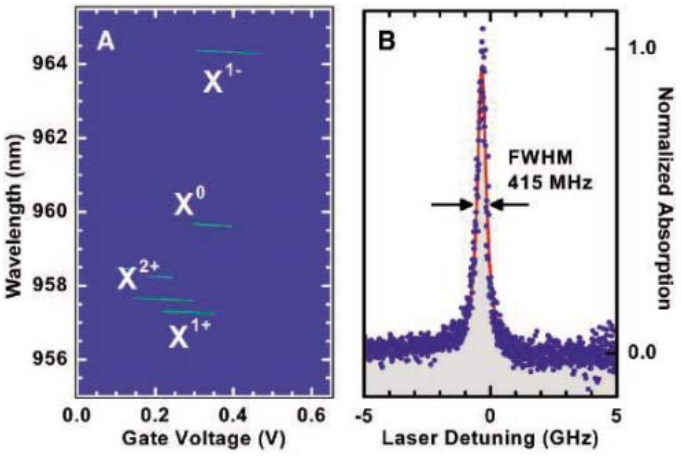


**Fig. 1.** (**A**) Photoluminescence from a single QD as a function of gate voltage. Each discrete jump in the emission spectrum corresponds to a charging state. Along with $X^0$ and $X^{1+(-)}$ lines, we observed a possible $X^{2+}$ line, which corresponds to optically charged double resident holes. (**B**) Absorption peak of a single-electron–charged QD at a fixed gate voltage. The probe laser power was 10 nW, corresponding to a Rabi frequency $\Omega_L = 0.25\ \Gamma$, and the absolute value of absorption was 0.4%. The full width at half maximum (FWHM) of the transition was 415 MHz.

or a magnetic field that is not parallel to the strong confinement ($z$) axis yields $\Gamma \gg \gamma \neq 0$, where $\Gamma$ and $\gamma$ are the allowed and forbidden spontaneous emission rates, respectively, as indicated in Fig. 2A. In addition to these optical transitions, the strong hyperfine interaction of the resident electron spin with the QD nuclear spin ensemble leads to random spin-flip events at rate $\xi_{\uparrow\downarrow}$. Previous studies on similar structures have shown that $\xi_{\uparrow\downarrow}^{(B\,=\,0)} \leq \Gamma$ in the absence of an external magnetic field $B$ but is strongly suppressed even under relatively weak magnetic fields ($B \sim 0.1$ T) because of incommensurate electron and nuclear Zeeman energies (*17*, *18*). This is the case depicted in Fig. 2B, where $\xi_{\uparrow\downarrow}^{(B\,>\,0)} \ll \gamma$. When a second re-pump laser is resonant with the other Zeeman-split trion transition $|\uparrow\downarrow, \blacktriangle\rangle$ — $|\uparrow\rangle$ (Fig. 2C), the $|\uparrow\rangle \rightarrow |\downarrow\rangle$ transition will also take place via spontaneous spin-flip Raman scattering, with a rate proportional to the laser intensity.

Figure 2D is the absorption analog of Fig. 1A, showing the expected $X^{1-}$ plateau at $B = 0$ T, where the QD is single-electron-charged for gate voltages ($V_{gate}$) in the 320- to 424-mV range. The probe laser was scanned across the $|\uparrow\downarrow, \blacktriangledown\rangle$ — $|\downarrow\rangle$ transition and had the corresponding circular polarization [$\sigma^{(-)}$] as determined by the optical selection rules. Figure 2E shows a suppression of the $X^{1-}$ plateau at a 0.2-T magnetic field: The QD becomes transparent for gate voltages in the 344- to 396-mV range. Given that the corresponding $X^0$ plateau remains unaffected under all magnetic fields, this strong suppression of the signal in the $X^{1-}$ plateau center is a signature of optical electron-spin pumping into the $|\uparrow\rangle$ state due to the unidirectional spontaneous Raman scattering process ($\gamma$) that dominates over the bidirectional spin-flip process ($\xi_{\uparrow\downarrow}^{(B\,>\,0)}$). In this case, we can confirm that the electron remained in the spin-up state 98.5% of the time, as the laser was resonant with the Pauli-blocked (*16*, *19*) $|\uparrow\downarrow, \blacktriangledown\rangle$ — $|\downarrow\rangle$ transition of the electronic spin-down state. A similar measurement at 0.3 T showed that the electron was in the $|\uparrow\rangle$ state 99.8% of the measurement time. To date, this value is the highest state-preparation fidelity reported in a solid-state system (*20*, *21*) and was achieved for both electron-spin states. The verification of higher-fidelity values is not limited by the physical mechanism involved but rather by our signal-to-noise level (*22*).

To prove that the electron was shelved in the $|\uparrow\rangle$ state by the probe laser at the $|\uparrow\downarrow, \blacktriangledown\rangle$ — $|\downarrow\rangle$ transition, we simultaneously applied a re-pump laser on the $|\uparrow\downarrow, \blacktriangle\rangle$ — $|\uparrow\rangle$ transition with orthogonal circular polarization [$\sigma^{(+)}$]. Figure 2F shows the resulting gate sweep, where an absorption peak at $V_{gate} = 372$ mV appears. The linewidth of this peak is equal to that shown in Fig. 1B, and it was observed when the re-pump laser was detuned from the probe laser by exactly 6 GHz; that is, the independently measured Zeeman splitting of the $X^{1-}$ transition. The fact that we recovered the probe laser absorption only when both lasers were resonant with the corresponding trion transitions indicates that the re-pump laser allowed for bidirectional spin-flip spontaneous Raman scattering and prohibited any net spin shelving: The system is now described by the illustration in Fig. 2C. When we kept the frequency of the re-pump laser unchanged but increased the magnetic field to 0.3 T, we observed that the absorption peak on the transparent section of the plateau shifted in accordance with the corresponding 9-GHz Zeeman splitting.

Figure 3A shows the magnetic-field dependence of the absorption displaying the Pauli blockade strength in the middle of the charging plateau, normalized to the maximum absorption at 0 T. Because $\Gamma$ and $\gamma$ have no magnetic-field dependence, our measurements reveal the magnetic-field dependence of the hyperfine-induced spin-flip rate $\xi_{\uparrow\downarrow}^{(B\,>\,0)}$. The red line is a theoretical curve obtained from rate equations by taking into account both photon-assisted hyperfine-induced spin-flip events and the spontaneous spin-flip Raman transition. The parameters used to simulate the experimental results are $\Gamma = 300$ MHz; $\gamma = 100$ kHz; the effective Overhauser field arising from randomly oriented nuclear spins ($B_{nuc}$) = $A/\sqrt{N} = 12.5$ mT (*23*); and the electronic $g$ factor ($g_e$) = –0.6 (*24*). The spin cooling rate (below saturation) is independent of laser power for the single-$\Lambda$ system, further supporting our assumption that both cooling and spin relaxation are (linearly) proportional to the intensity of the probe laser. We emphasize, however, that an exponential fit seems to be in better agreement with the data, indicating that our simple theoretical model may not be capturing other relevant processes such as phonon-assisted spin-flips or spin-orbit coupling.

The triangles at 0.2 T in Fig. 3A correspond to the partial (full) recovery of the absorption as the bidirectional optical spin pumping was realized using the re-pump laser, which was of weaker (comparable) intensity with respect to the probe laser. The state-preparation fidelity for the electron spin as a function of magnetic field is plotted in Fig. 3B. At 0.3 T, the electron is already in the spin-up state, with a fidelity exceeding 99.8%. With each data point, the corresponding spin temperature is provided as obtained from the state occupancies, and a net cooling from 4.2 to 20 mK is achieved. As a token of our cooling efficiency, we emphasize that such state-preparation fidelity can be achieved only at a 62-T external field, when one relies solely on thermal equilibration at 4.2 K in the absence of laser cooling.

Another striking feature of the gate sweep depicted in Fig. 2E is that the absorption remains essentially unaffected for gate voltages that define the edges of the plateau. Spin cooling is ineffective in this regime despite the fact that the hyperfine-induced electron spin-flip rate should not depend on the gate voltage. On the other hand, it has been shown that the cotunneling-induced spin-flip rate varies across the absorption plateau by a factor as large as $10^6$ (*25*) for 20-meV electron charging energy (*26*). We expect the electron spin-flip rate $\xi_{\uparrow\downarrow}^{(B)}$ to be dominated by cotunneling at the edges of the plateau, leading to a suppression of spin pumping. In more general terms, the spin-cooling dynamics is determined by interplay between spontaneous spin-flip Raman scattering, hyperfine-induced electron spin-flips, and electronic cotunneling processes. In the absence of magnetic field and at a gate voltage within the plateau middle, QD spin strongly interacts with the QD nuclei. Alternatively, at finite magnetic field and at a gate voltage within the edges of the plateau, QD spin strongly interacts with the back-gate electron reservoir. In both regimes, the electron spin cannot be considered as an isolated quantum system any more because of its dominant coupling to a spin or a charge reservoir.

The next step after gaining a full understanding of and control over such spin dynamics will be to

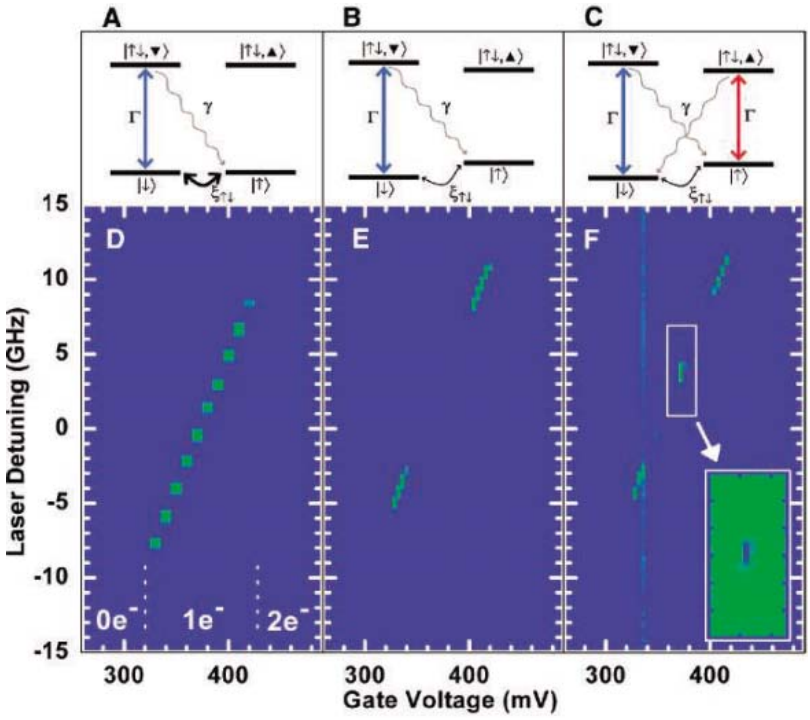


**Fig. 2.** (**A**) Four-level scheme illustrating the ground and excited states of a single-electron–charged QD. Because $\xi_{\uparrow\downarrow}^{(B=0)}$ is relatively strong when the spin states are degenerate, the optical transitions do not alter spin-state occupancies. (**B**) When the degeneracy is lifted by a magnetic field, the optical transitions form a $\Lambda$ system, which in turn allows for spin cooling via spin-flip spontaneous Raman transition under the condition $\Gamma \gg \gamma \gg \xi_{\uparrow\downarrow}$. (**C**) A symmetric double-$\Lambda$ system allows for bidirectional spin pumping, and therefore manipulation of spin-state occupancies can be achieved as a function of laser intensity ratios. (**D**) Absorption as a function of probe-laser frequency and 10-mV gate voltage increments in the absence of a magnetic field. The slope is determined by the DC-Stark shift. Absorption is constant throughout the voltage plateau, which identifies the range for single-electron charging of the QD. (**E**) Same plateau with finer (4-mV) gate voltage increments under a 0.2-T magnetic field. The plateau middle is suppressed as spin cooling takes effect and induces strong Pauli blockade on the investigated transition. Indeed, we nearly achieved $10^3$-fold suppression. The remaining absorption at the plateau edges coincides with the strong cotunneling regime. The spectral displacement of the plateau is due to the diamagnetic and Zeeman shifts, as imprinted in the 4-GHz detuning from the probe laser. (**F**) Same plateau in the presence of a re-pump laser detuned by 6 GHz with respect to the probe laser. Ultra–high-fidelity spin cooling is still observed, except when both lasers are simultaneously resonant with the 6-GHz-split Zeeman transitions. At this point, the double-$\Lambda$ system of (C) is realized, and the sharp resonance peak within the suppressed part of the plateau is the confirmation for this realization. (Inset) A finer scan of the indicated rectangle; each tick on the *y* axis corresponds to 1 GHz.

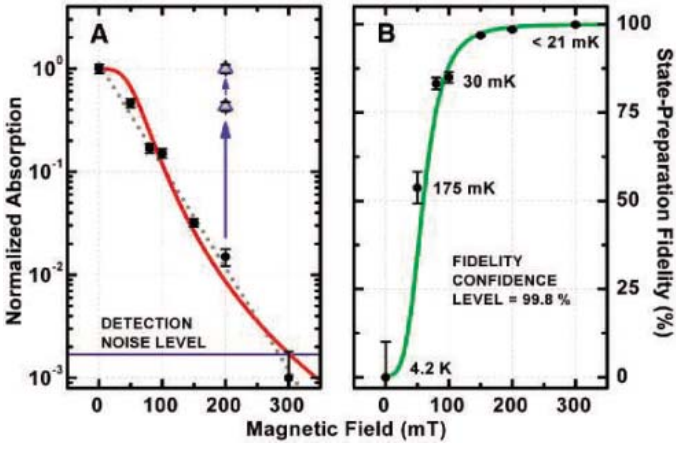


**Fig. 3.** (**A**) Magnetic field dependence of the absorption at $V_{gate}$ = 372 mV. A nearly $10^3$-fold suppression is obtained because of Pauli blockade in the single-$\Lambda$ scheme. In the double-$\Lambda$ scheme, absorption depends on the intensity ratio of the two lasers. The triangles indicate the partial (full) recovery of the absorption signal when $P_{re\text{-}pump} = 0.25\ P_{probe}$ ($P_{re\text{-}pump} = P_{probe}$). (**B**) State-preparation fidelity with increasing magnetic field. At 0.3 T, the electron is already prepared in the spin-up state with at least 99.8% fidelity. The electron-spin temperature is provided for each data point.

tune the spin-flip Raman transition rate as a function of magnetic field orientation with respect to the strong confinement axis. Our results constitute the first step toward stimulated Raman transition (*27*) on a single QD electron for coherent preparation of an arbitrary superposition of spin states, as well as cavity-assisted spin-flip Raman transitions as a source of indistinguishable single photons with near-unity collection efficiency (*28*). The electron-spin cooling mechanism described here can alternatively be used for dynamical nuclear-spin polarization.

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# Optical Spectroscopy of Individual Single-Walled Carbon Nanotubes of Defined Chiral Structure

Matthew Y. Sfeir,[1,6]* Tobias Beetz,[5] Feng Wang,[2] Limin Huang,[3] X. M. Henry Huang,[4] Mingyuan Huang,[4] J. Hone,[4] Stephen O'Brien,[3] J. A. Misewich,[6] Tony F. Heinz,[2] Lijun Wu,[5] Yimei Zhu,[5] Louis E. Brus[1]

We simultaneously determined the physical structure and optical transition energies of individual single-walled carbon nanotubes by combining electron diffraction with Rayleigh scattering spectroscopy. These results test fundamental features of the excited electronic states of carbon nanotubes. We directly verified the systematic changes in transition energies of semiconducting nanotubes as a function of their chirality and observed predicted energy splittings of optical transitions in metallic nanotubes.

The electronic and optical properties of single-walled carbon nanotubes (SWNTs) depend sensitively on atomic structure, with distinctive energy-level structures corresponding to both metallic and semiconducting species (*1*–*4*). Here, we report results of an investigation in which high-sensitivity optical spectroscopy (*5*) and electron diffraction (ED) are combined to examine individual SWNTs. Simultaneous use of these two different experimental techniques at the single nanotube level has permitted the measurement of electronic transitions in individual SWNTs of fully and independently determined physical structure. Recent studies on the vibrational structure of individual SWNTs have resulted from a similar combination of direct structural analysis and Raman spectroscopy (*6*). These data permit us to test key theoretical constructs concerning the electronic states in SWNTs. For semiconducting SWNTs, we verify the family behavior of the transition energies as a function of their precise atomic structure or chirality. The validity of this prediction is important not only for fundamental understanding of the electronic properties of SWNTs but also because of its critical role in guiding spectroscopic assignments (*7*–*10*). We also observe splitting of optical transitions in metallic SWNTs. Both this result and family behavior, initially predicted from simple tight-binding zone-folding models, are found to survive in actual SWNTs despite the presence of curvature effects (*11*) and strong multicarrier interactions (*12*–*19*).

The physical structure of SWNTs can be viewed as the consequence of rolling a graphene sheet of $sp^2$-hybridized carbon atoms into the desired structure. The chiral vector $\mathbf{na_1} + \mathbf{ma_2}$ is correspondingly used as a label for the atomic structure, where the integers $n$ and $m$ denote the number of steps along the graphene basis vectors, $\mathbf{a}_{1,2}$, to reach folding points. The chiral indices $(n,m)$ define both the diameter $d_t$ of the nanotube and the chiral angle θ of the hexagons of carbon atoms wrapped around its circumference. Rolling up a graphene sheet leads to zone-folding in momentum space to satisfy the relevant periodic boundary conditions (Fig. 1). From the meeting at the $K$ and $K'$ points in the Brillouin zone of the valence and conduction bands in the zero band-gap graphene structure, one predicts metallic SWNTs for mod($n$-$m$, 3) = 0 (Fig. 1A) and semiconducting SWNTs for mod($n$-$m$, 3) = 1, 2 (Fig. 1, B and C). Within an approximation of linear and isotropic dispersion of the graphene bands near the $K$ and $K'$ points, the band-edge energies in SWNTs scale simply as $1/d_t$.

The experimentally observed dependence of band structure and transition energies of nanotubes on their precise chiral structure originates from the deviation from the linearity of the graphene bands and their variation with directions in the Brillouin zone. This effect, referred to as the trigonal warping (*20*–*22*), leads to two general predictions within the context of a noninteracting electronic structure model. The first, known as "family behavior," is a systematic pattern of chirality dependence of the optical transition energies in semiconducting SWNTs according to whether mod($n$-$m$, 3) = 1 or 2. Although never directly tested, this predicted pattern in the nanotube transition energies has been widely used in making structural assignments of the measured spectroscopic features (*7*–*10*). The present research provides a firm experimental basis for the predicted family variation with nanotube chirality. The second prediction is a splitting of the electronic transitions for nonarmchair (θ ≠ 30°) metallic nanotubes. This phenomenon has not yet been observed. Indeed, recent measurements of Raman excitation profiles reveal only a single peak (*9*, *23*). Although both of these predictions follow from qualitative features of the graphene band structure, their validity is not assured because of the existence of important corrections, including the effect of curvature on nanotube properties (*11*) and the influence of the strong electron-electron interactions in these quasi–one-dimensional (1D) structures (*12*–*18*). Here, we test these predictions directly using optical measurements of individual nanotubes.

The SWNTs investigated in this study are grown by chemical vapor deposition with a cobalt catalyst on a custom-designed silicon substrate (*24*). The silicon substrate is prepared with a 25 μm × 1 mm open slit, over which the nanotubes are suspended. The growth condi-

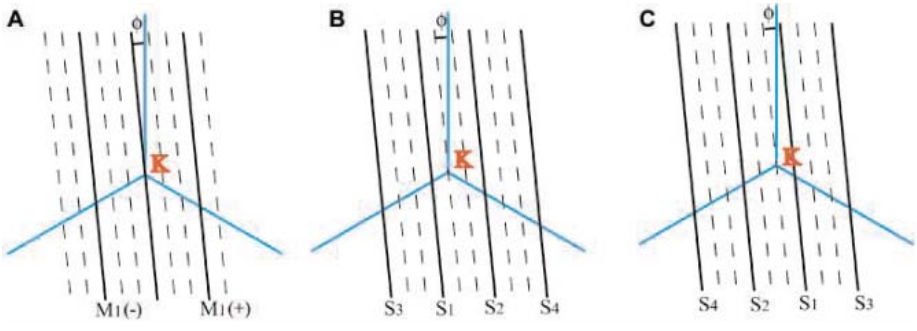


**Fig. 1.** Zone-folding picture of carbon nanotubes around the $K$ point in the Brillouin zone of graphene. The blue lines are the Brillouin zone boundary, and the angle ϕ is the deviation of the nanotube from the armchair direction. The solid lines spaced by a distance $2/d_t$ represent the available states consistent with the boundary conditions for (**A**) metallic nanotubes with mod($n$-$m$, 3) = 0 and semiconducting nanotubes with (**B**) mod($n$-$m$, 3) = 1 and (**C**) mod($n$-$m$, 3) = 2. The dashed lines spaced by $2/3d_t$ have been added to clarify the relation between the different classes. Due to the asymmetry on the two sides of $K$ point for finite ϕ, nonarmchair metallic tubes have two $M_{1(\pm)}$ bands with differing energies. The same asymmetry leads to a systematic variation of transitions energies for semiconducting tubes of different chirality: Odd bands ($S_1$, $S_3$, ..) in (B) have lower energies than their counterparts in (C) for tubes of the same diameter, whereas even bands ($S_2$, $S_4$, …) have higher energies. The differences increase with the asymmetry level and the angle ϕ. These trends give rise to the family behavior in SWNTs.

[1]Department of Chemistry, [2]Department of Physics and Electrical Engineering, [3]Department of Applied Physics and Applied Mathematics, [4]Department of Mechanical Engineering, Columbia University, 3000 Broadway, New York, NY 10027, USA. [5]Center for Functional Nanomaterials, [6]Condensed Matter Physics and Materials Science, Brookhaven National Laboratory, Upton, NY 11973, USA.

*To whom correspondence should be addressed. E-mail: msfeir@bnl.gov

tions are adjusted so that the nanotubes are separated from one another by an average distance of 100 μm. This arrangement allows us to investigate the nanotubes both optically and by electron diffraction without further modification. The electronic transitions of the individual SWNTs are probed by Rayleigh scattering spectroscopy (*5*). This method, which uses elastic light scattering from a continuum light source, provides high-quality spectra of individual SWNTs with short data collection times. The optical spectra were collected with a tightly focused probe beam, so we could determine the position of each nanotube with micron spatial precision. The sample was then transferred to a JEOL 3000F transmission electron microscope (TEM) for structure determination. While high-resolution TEM imaging was useful to estimate the nanotube diameter and verify its single-walled character, detailed structural analysis was performed by electron diffraction. For this purpose, a parallel 20-nm-diameter electron beam was used to illuminate the sample, with the nanotube axis carefully aligned to a position normal to the beam; an electron energy of 200 keV was used to minimize radiation damage during exposure. The ED pattern was recorded on a DITABIS image plate system that provided the high dynamic range (20 bit) needed for making accurate structure assignments. The structure determination of each SWNT is performed in two steps.

An initial analysis of the ED patterns with a method similar to that of Gao *et al*. (*25*) yielded a set of possible structures for each SWNT. A unique structural assignment was then obtained by a quantitative comparison of the experimental ED pattern with simulated ED patterns for all such candidate structures, as shown in Fig. 2, A and B, for a (16,11) SWNT; the corresponding nanotube structure is illustrated in Fig. 2C. The Rayleigh scattering spectrum of this SWNT is presented in Fig. 3A. The results of such structural analysis and simultaneous measurement of the optical transition energies for a set of semiconducting and metallic nanotubes are summarized in Table 1. Data for separate observations of two different (10,10) SWNTs demonstrate the high reproducibility of the procedure.

With independent information on the structure and optical spectra of SWNTs, we can directly examine their relation. The semiconducting species are labeled by their optical transitions $S_{ii}$ with index $i$ corresponding to the transition order. Shown in Fig. 3A are the Rayleigh spectra of the $S_{33}$ and $S_{44}$ transitions for (16,11) and (15,10) SWNTs. These two species have similar chiral angles (23.9° and 23.4°) but different diameters (1.83 and 1.71 nm). For this case, the decrease in diameter of (15,10) SWNT compared with the (16,11) SWNT yields a blue shifts in the transition energies of ~150 meV but very little change in the ratio of the $S_{44}$ and $S_{33}$ transition energies (1.135 versus 1.150, for the smaller and larger diameters, respectively). The variation in the transition energies of the corresponding features in the two SWNT species is close to the simple $(1/d_t)$ scaling expected when chirality effects are neglected.

The dependence of the optical transition energy on the SWNT chiral angle is expected to show well-defined trends. The most noteworthy feature for the semiconducting SWNTs, as discussed above, is the family behavior that distinguishes the mod-1 and mod-2 SWNT based on the value of mod ($n$-$m$, 3). As depicted in Fig. 1, B and C, the corresponding transitions for mod-1 and mod-2 nanotubes lie on different sides of the $K$ point. Because of the trigonal symmetry of the Brillouin zone, the bands on the right side of $K$ have higher energies compared with those lying at the same distance from the $K$ point on the left side. This asymmetry increases with the angle $\phi \equiv 30° - \theta$, measuring the deviation of the chiral tubes from the armchair direction. As such, the mod-1 tubes have smaller energies than mod-2 tubes of the comparable diameter for odd transitions ($S_{11}$, $S_{33}$,…) and larger energies for even transitions ($S_{22}$, $S_{44}$, …). The deviation from the average value becomes greater for tubes with increasing $\phi$ (decreasing chiral angle $\theta$) but with opposite excursions for mod-1 and mod-2 tubes. These expected trends are summarized in the relation $S_{even}^{\text{small }\theta,\text{ mod-1}} > S_{even}^{\text{large }\theta,\text{ mod-1}} > S_{even}^{\text{large }\theta,\text{ mod-2}} > S_{even}^{\text{small }\theta,\text{ mod-2}}$. An analogous relation applies for odd transitions, with the order of the inequalities reversed. To reduce the dependence on the diameter of the nanotube and isolate the chiral angle dependence, we consider the ratio of energies for the even and odd transitions. Just this approach was applied in the structure-assignment scheme of Bachilo *et al*. (*7*). In terms of this ratio of transition energies, the family behavior is described by $(S_{even}/S_{odd})^{\text{small }\theta,\text{ mod-1}} > (S_{even}/S_{odd})^{\text{large }\theta,\text{ mod-1}} > (S_{even}/S_{odd})^{\text{large }\theta,\text{ mod-2}} > (S_{even}/S_{odd})^{\text{small }\theta,\text{ mod-2}}$.

We now examine the validity of these general predictions using our optical data for the $S_{33}$ and $S_{44}$ transitions in SWNTs of independently determined structure. Rayleigh scattering spectra for (13,12) and (15,10) SWNTs are presented in Fig. 3B. These nanotubes have almost identical diameters of 1.70 and 1.71 nm, respectively, and provide an ideal case to probe the effect of chiral angle on transition energy. We observe that the mod-1 (13,12) SWNT has a smaller transition energy than the mod-2 (15,10) SWNT for the $S_{33}$ resonance and larger transition energy for $S_{44}$, in precise accordance with the predicted family behavior. To compare all four

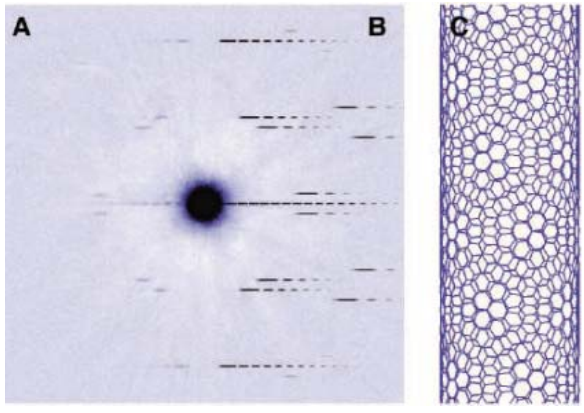


**Fig. 2.** Measurement of the carbon nanotube chiral indices. (**A**) Experimental TEM diffraction image from a semiconducting (16,11) SWNT and (**B**) the corresponding simulated diffraction pattern. (**C**) Model of the structure of the (16,11) SWNT.

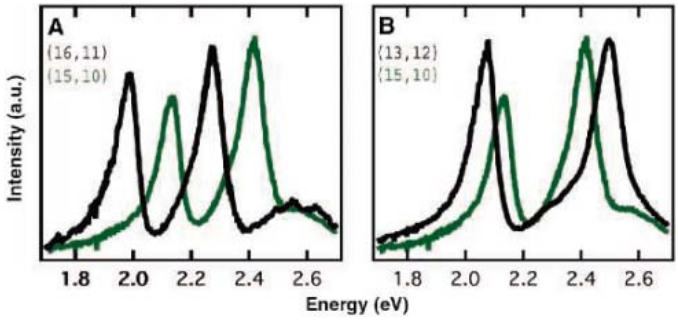


**Fig. 3.** Measuring chirality and diameter effects in semiconducting SWNTs. (**A**) Comparison of the Rayleigh scattering spectra for the $S_{33}$ and $S_{44}$ transitions of the (16,11) and a (15,10) SWNTs. These SWNTs have chiral angles differing by only 0.5°, but diameters of 1.83 and 1.71 nm, respectively. We see an upward shift of both transition energies for the smaller diameter tube but little change in the ratio of the $S_{44}/S_{33}$ transition energies. (**B**) Comparison of a (13,12) and a (15,10) SWNT. These SWNTs have nearly identical diameters, but chiral angles of 28.7° and 23.4°, respectively. In this case, the average transition energies for the $S_{33}$ and $S_{44}$ transitions in the two structures are almost identical. The ratios of the transition energies clearly differ and obey the family relation discussion in the text.

semiconducting SWNTs, we calculate the ratios of the $S_{44}/S_{33}$ transition energies. For (13,12), (13,11), (16,11), and (15,10) tubes, which are (mod-1, θ = 28.7°), (mod-2, θ = 27.2°), (mod-2, θ = 23.9°), and (mod-2, θ = 23.4°), respectively, we obtain $S_{44}/S_{33}$ ratios of 1.21 > 1.17 > 1.15 > 1.13. The experimental results are in exact agreement with the pattern predicted by family behavior. This finding is far from obvious given the deficiencies in the tight-binding model on which the predictions are based. In particular, the tight-binding model fails to capture the strong many-body effects (*12*–*19*) that are present in such 1D systems, as well as the effect of the finite curvature of the SWNTs (*11*, *26*). Indeed, the experimental results differ from the tight-binding predictions (*1*, *20*–*22*) for both the absolute transition energies and their ratios (1.24, 1.22, 1.20, and 1.19, respectively). The errors in the predicted transition energies in the tight-binding calculation, although substantial, apparently display only weak chirality dependence. This leaves the predicted family behavior intact.

We now turn to investigation of metallic SWNTs with optical transitions labeled by $M_{ii}$. Because the metallic nanotubes do not fluoresce, much less is known about their optical properties. Most of the available data on these SWNTs have been obtained from resonance Raman scattering spectroscopy (*27*, *28*), and recent investigations have produced puzzling results (*9*, *23*). Although the zone-folding picture clearly predicts a splitting of energy for the $M_{ii}$ transitions for any nonarmchair nanotubes, only one of two peaks has been observed in all reported data.

The Rayleigh spectrum of the (10,10) armchair SWNT (Fig. 4A) exhibits a well-defined single $M_{11}$ resonance feature, as expected, because no splitting is predicted for such high-symmetry structures. As an example of a chiral metallic structure, we present the spectrum of an (11,8) SWNT (Fig. 4B). We see that the $M_{11}$ transition is indeed split into two separate peaks (Fig. 1A), as predicted in single-particle treatments of the SWNT electronic states (*20*–*22*, *29*). We have also observed a splitting of the optical transitions for higher order metallic transitions. The spectrum (Fig. 4C) for a (20,14) SWNT displays an $M_{22}$ feature split into two features separated by 110 meV. These data provide the experimental evidence of such splitting of the electronic states in metallic nanotubes. The inability to identify both members of the pair of split optical transitions in the Raman excitation spectroscopy has been attributed to the different electron-phonon coupling strength for the two branches of the split transition (*9*, *18*). This complicating factor does not exist for the Rayleigh spectroscopy used in our measurement. The elastic Rayleigh scattering features are determined directly by the strength of optical transitions.

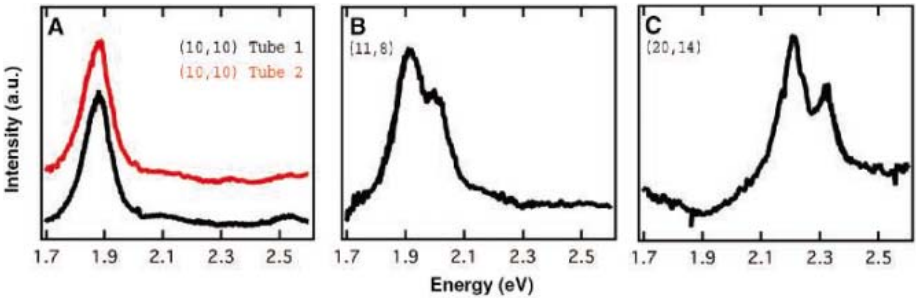


**Fig. 4.** Observation of the trigonal warping effect in metallic SWNTs. (**A**) Rayleigh scattering spectrum (black) for the $M_{11}$ transition in the (10,10) SWNT. A spectrum (red and displaced upwards) for a different (10,10) SWNT was obtained in a separate experiment. (**B**) Spectrum for an (11,8) SWNT. The $M_{11}$ transition is clearly split into two peaks, as predicted by the trigonal warping effect. (**C**) Spectrum for the (20,14) SWNT showing the splitting of the $M_{22}$ transition.

**Table 1.** Physical and spectroscopic data from several SWNTs analyzed by electron diffraction and Rayleigh scattering measurements on the same nanotube. The SWNT structures are described by their chiral indices (*n*,*m*) and the corresponding diameter $d_t$ and chiral angle θ. The energies of the electronic transitions (with an estimated uncertainty of 10 meV) are obtained from fitting the Rayleigh spectra of each SWNT.

| (*n*,*m*) | mod(*n*-*m*, 3) | $d_t$ (nm) | θ (°) | Transition | Eii (eV) |
|---|---|---|---|---|---|
| (16,11) | 2 | 1.83 | 23.9 | $S_{33}$ | 2.00 |
| | | | | $S_{44}$ | 2.30 |
| (15,10) | 2 | 1.71 | 23.4 | $S_{33}$ | 2.15 |
| | | | | $S_{44}$ | 2.44 |
| (13,12) | 1 | 1.70 | 28.7 | $S_{33}$ | 2.09 |
| | | | | $S_{44}$ | 2.52 |
| (13,11) | 2 | 1.63 | 27.2 | $S_{33}$ | 2.19 |
| | | | | $S_{44}$ | 2.56 |
| (10,10) | 0 | 1.36 | 30 | $M_{11}$ | 1.93 |
| (11,8) | 0 | 1.30 | 24.8 | $M_{11(-)}$ | 1.93 |
| | | | | $M_{11(+)}$ | 2.02 |
| (20,14) | 0 | 2.35 | 24.2 | $M_{22(-)}$ | 2.22 |
| | | | | $M_{22(+)}$ | 2.36 |


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# Biologically Inspired Artificial Compound Eyes

Ki-Hun Jeong, Jaeyoun Kim, Luke P. Lee*

This work presents the fabrication of biologically inspired artificial compound eyes. The artificial ommatidium, like that of an insect's compound eyes, consists of a refractive polymer microlens, a light-guiding polymer cone, and a self-aligned waveguide to collect light with a small angular acceptance. The ommatidia are omnidirectionally arranged along a hemispherical polymer dome such that they provide a wide field of view similar to that of a natural compound eye. The spherical configuration of the microlenses is accomplished by reconfigurable microtemplating, that is, polymer replication using the deformed elastomer membrane with microlens patterns. The formation of polymer waveguides self-aligned with microlenses is also realized by a self-writing process in a photosensitive polymer resin. The angular acceptance is directly measured by three-dimensional optical sectioning with a confocal microscope, and the detailed optical characteristics are studied in comparison with a natural compound eye.

Compound eyes in nature present intriguing topics in physiological optics because of their unique optical scheme for imaging. For example, a bee's eye has thousands of integrated optical units called ommatidia spherically arranged along a curvilinear surface so that each unit points in a different direction (Fig. 1A). Each ommatidium consists of a light-diffracting facet lens, a crystalline cone, and photoreceptor cells with a wave-guiding rhabdom (*1*–*3*) (Fig. 1B). The omnidirectionally arranged ommatidium collects incident light with a narrow range of angular acceptance and independently contributes to the capability of wide field-of-view (FOV) detection (*4*–*6*).

Artificial implementation of compound eyes has attracted a great deal of research interest, because the wide FOV exhibits a huge potential for medical, industrial, and military applications. So far, imaging with a FOV over 90° has been achieved only with fish eye lenses, which rely on bulky and expensive multiple lenses and require stringent alignment. The use of miniaturized, arrayed optical components fabricated by using semiconductor planar processing technologies has been proposed to simultaneously mimic the structure and function of an individual ommatidium and the large-scale collection of ommatidia. The imaging systems using microlens arrays (*7*, *8*) or graded index rod arrays (*9*, *10*) in combination with matching pinhole arrays are good examples. More biomimetic efforts to implement artificial compound eyes were reviewed in (*11*) along with an outline of biological imaging systems. Achieving a wide FOV in those structures, however, has been hindered mainly by the inherent flatness of the arrayed optical components. In addition, the need to align multiple layers of arrayed components during assembly of the above-mentioned imaging systems gives them no advantage over fish eye lenses. For practical implementations of compound eyes with wide FOV, the requirement of curvature-compatible, self-aligned fabrications schemes is evident.

In this work, biologically inspired artificial compound eyes were developed in a small form factor with three-dimensional (3D) configurations. These biomimetic compound eyes are anatomically as well as functionally close to natural compound eyes (Fig. 1C). The artificial ommatidium consists of a honeycomb-packed hexagonal microlens with a low Fresnel number ($N_F < 10$), a cuvette-shaped polymer cone, and a polymer waveguide that has a higher index solid core surrounded by a lower index solid cladding in the polymer resin (Fig. 1D). Three-dimensional polymer synthesis of an artificial compound eye can be realized through microlens templating, reconfigurable microtemplating, and self-writing in a photosensitive polymer resin. Each ommatidium was omnidirectionally arranged in a hemispherical polymer dome. Like the crystalline cone in nature, the polymer cone helps guide the focused light into the polymer waveguide, and subsequently the guided light arrives at the end of the waveguide core (*12*). Lastly, light detection can be done by photodetector arrays. In 3D implementation, microlens-assisted self-writing and polymer replication processes were used to minimize the lens-waveguide coupling loss and to realize a spherical configuration, respectively.

Polymer synthesis of artificial ommatidia can be done by using a microlens-assisted self-writing of waveguides and two cross-linking mechanisms in a photosensitive polymer resin (*13*) (Fig. 2A). Ultraviolet (UV) light was focused through low $N_F$ microlenses molded by a photosensitive polymer resin and was self-trapped after passing the focal

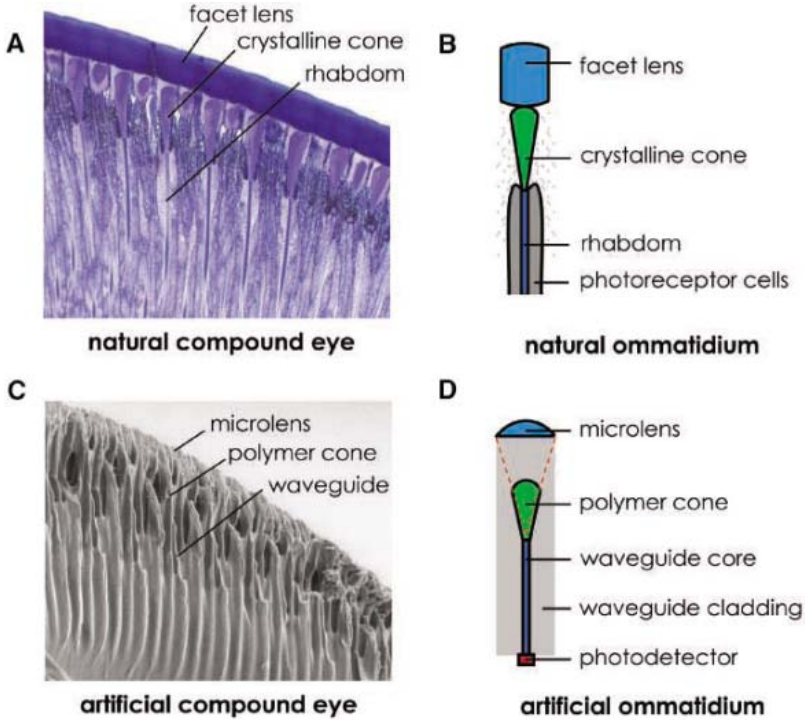


**Fig. 1.** Anatomical comparisons between a natural compound eye and an artificial compound eye described from the cross sections. (**A**) An optical micrograph of a honeybee's apposition compound eye (courtesy of B. Greiner). As an individual optical unit, (**B**) a natural ommatidium consists of a facet lens, a crystalline cone, and photoreceptor cells with a wave-guiding rhabdom. (**C**) A scanning electron micrograph of an artificial compound eye and (**D**) an artificial ommatidium comprising a microlens, a polymer cone, and an optical waveguide that has a higher index core surrounded by a lower index cladding in a polymer resin. Light impinging onto a microlens is coupled with polymer cones and waveguides and then guided to the end of the waveguide.

Biomolecular Nanotechnology Center, Berkeley Sensor and Actuator Center, Department of Bioengineering, 485 Evans Hall No. 1762, University of California, Berkeley, CA 94720, USA.

*To whom correspondence should be addressed. E-mail: lplee@socrates.berkeley.edu

plane because of the refractive index change by the photopolymerization (*14–18*).

The exposed portion above threshold energy for photopolymerization was photocross-linked by postbaking. The underexposed portion below threshold energy was still UV sensitive but was thermally cross-linked by heating above the temperature where a photoacid generator (PAG) in the photosensitive polymer resin starts to degrade. At that point, the unexposed portion became insensitive to additional UV light. In the experiment, a commercialized negative tone photoresist (SU-8, Microchem Corporation, Newton, MA) was used as a photosensitive polymer resin. Initially the refractive index of an SU-8 monomer in a liquid phase, measured by an Abbe refractometer (ARIAS 500, Reichert, Incorporated, Depew, NY), was $n_{monomer}$ = 1.550. The index of a 1.5-μm-thick thin monomer film, prepared by spincoating and a soft bake, increased to 1.584 because of the evaporation of SU-8 solvent, that is, gamma butyrolactone (GBL). After UV exposure of 900 mJ/cm$^2$ and a postexposure bake, the index change measured by a spectroscopic ellipsometer increases up to $\Delta n_{photo}$ = 0.021, and the fully photocross-linked SU-8 index was $n_{photo}$ = 1.605. After thermal cross-linking, the index of the exposed portion fully cross-linked by UV was constant, but that of the unexposed portion decreased by 0.008. Consequently, the maximum index change between both cross-linking core and cladding eventually turned out to be $\Delta n_{SU\text{-}8}$ = 0.029.

The formation of the self-written waveguide during UV exposure was simulated by using a fast Fourier transform–based beam propagation method (Fig. 2B). In the simulation, the propagating exposure beam, while being diffracted by the index distribution, imparts photon energy to the photosensitive medium and modifies its refractive index as well. The modified refractive index profile was used to simulate the next round of propagation, and so on. The imparted energy, or the irradiation dose, $E$, at one location has been calculated as the product of the field intensity at that point and the unit time duration. The increase in the refractive index is approximated to be linear between the initial and the saturated indices. The microlens first focuses the exposure beam with about 50 μm of back focal length. The initial beam intensity and the unit time duration have been iteratively optimized to initiate the self-writing process from the focal point. The relatively large refractive index contrast of the photosensitive resin facilitated the formation of a straight, over-100-μm-long waveguide. The "diffusion" of the refractive index due to the chemically amplifying nature of the photosensitive resin (SU-8) was ignored in this simulation. As a result, the simulated waveguide was thinner than the one obtained experimentally. The rough surface of the simulated waveguide, in contrast to the smooth surface of the actual self-written waveguides, can also be ascribed to the exclusion of the diffusion effect. The combined action of the high index contrast and the diffusive, self-smoothing index profile was required to improve the efficiency of the self-writing process. Other than that, the simulated index profiles taken when $E$ reaches 5 to 20 times the value of the cross-linking threshold, $E_{th}$, exhibited good qualitative agreements with the observed waveguide structures. In our experiment, with the use of the previous method, the formation of large-scale artificial ommatidia self-written by 300-μm microlenses depended on UV exposure energy. It turned out that the formation of a polymer cone occurs after that of a waveguide core as UV exposure energy increases (Fig. 2C). At the level of hexagonal microlens of 25 μm in diameter, the formation of polymer cones and waveguide cores was also visualized by dark-field optical microscopy (Fig. 2D). The visualization was accomplished by dissolving unexposed portions in a solvent before thermal cross-linking. Polymer cones and waveguide cores were placed on a substrate because of the high aspect ratio of core diameter to core length.

The light guiding (λ = 635 nm) through artificial ommatidia has also been demonstrated by optical sectioning along the optical axis with a laser scanning transmission confocal microscope. An artificial ommatidium after thermal cross-linking (microlens with $F$ number of 1.93 ($F$/1.93), lens diameter of 30 μm ($D_L$ = 30 μm), and $N_F$ = 7.3; index difference between waveguide core and cladding was 0.029) showed

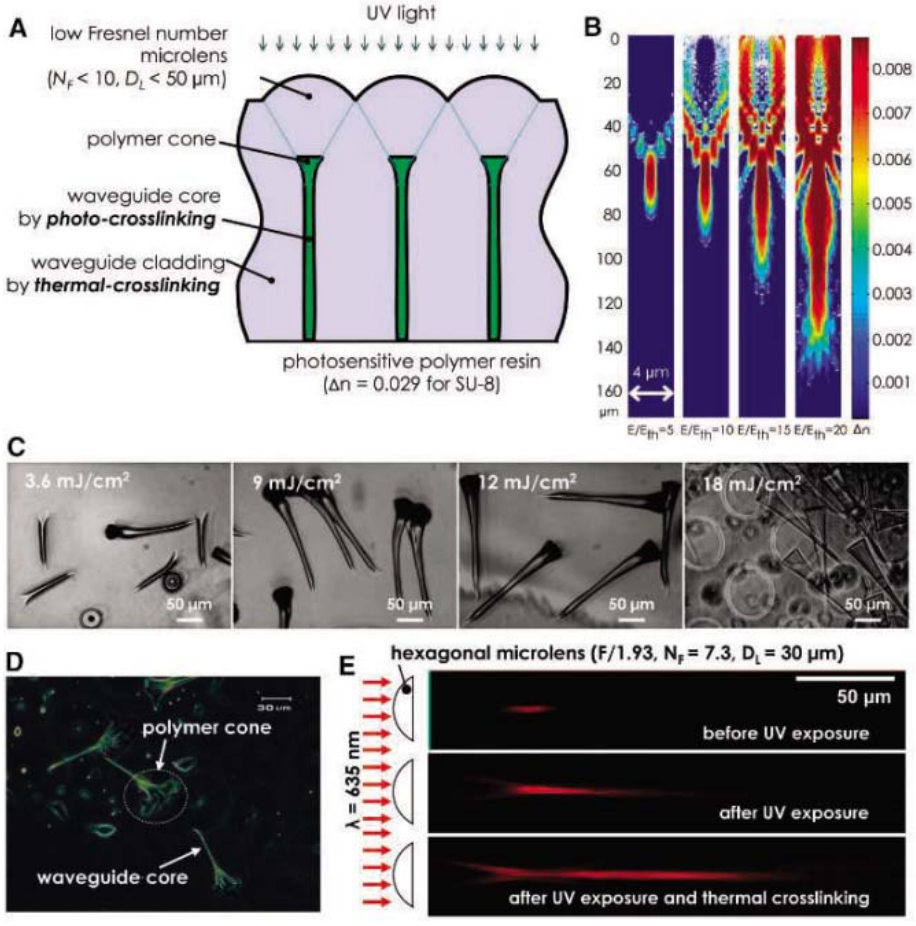


**Fig. 2.** Polymer synthesis of artificial ommatidia. (**A**) Two-step cross-linking mechanisms, that is, photocross-linking for waveguide cores and thermal cross-linking for waveguide claddings. (**B**) Simulated refractive index distributions of polymer waveguides formed by microlens-assisted self-writing by four different values of $E$. $E_{th}$ is the threshold irradiation dose that can initiate the cross-linking process in the polymer. (**C**) The formation of polymer cones and waveguide cores self-written by 300-μm-diameter microlenses depending on UV exposure. (**D**) A dark-field micrograph of polymer cones and waveguide cores placed on a substrate after completely dissolving unexposed portions in a solvent before thermal cross-linking. Note that the waveguide cores fall down because of the high aspect ratio of core diameter to core length. (**E**) Optically sectioned confocal micrographs of light at 635 nm coupled through an artificial ommatidium before UV exposure (only microlens), after UV exposure (waveguide core by photocross-linking), and after UV exposure and thermal cross-linking.

strong light guiding in comparison with only a microlens or with only UV photopolymerization (Fig. 2E).

The spherical configuration of artificial ommatidia can be achieved through a polymer replication process by reconfigurable microtemplating, that is, the polymer replication using the deformed elastomer membrane with microlens patterns (*19*) and self-written waveguides with a lens-assisted UV exposure for self-written waveguides. Honeycomb-packed hexagonal photoresist microlens arrays were prepared on a silicon substrate (Fig. 3A), and the lens template was molded onto a 22-μm-thick slab of polydimethylsiloxane (PDMS) elastomer (Fig. 3B). For reconfigurable microtemplating, a 5-mm-thick PDMS elastomer slab with a microfluidic channel and a circular through-hole 2.5 mm in diameter perforated by mechanical punching was permanently bonded to a 22-μm-thick PDMS replica of concave microlenses after an oxygen plasma surface treatment. The microlens replica was then released from the microlens template (Fig. 3C). Negative air pressure ranging from 5 to 30 kPa was applied through a microfluidic channel to deform the PDMS membrane with concave microlenses (Fig. 3D). A solvent-free UV-curable epoxy resin (NOA 68, Norland Products Incorporated, Cranbury, NJ) was precisely dispensed onto the deformed elastomer membrane, covered with a glass coverslip, and then fully cross-linked for 2 hours with UV light of 0.5 mW/cm$^2$ (Fig. 3E). For a batch replication, a 3D master mold was prepared with a five-by-five array of the 3D epoxy resin replicas with different curvatures glued on a Petri dish, and the master mold was again replicated with PDMS (Fig. 3F). The pattern polarity of the 3D PDMS replica was reversed by molding it with a commercial photosensitive polymer resin (NANO SU-8, formulated in cyclopentanone). The volume of 40 μl was precisely dispensed in each concave dome and prebaked at 120°C for 20 min to remove the solvent. An additional prebake process was also carried out at 120°C for 1 hour right after covering each droplet with a 10-mm-diameter circular glass (Fig. 3G). The SU-8 replica with convex microlenses along the circumference kept its shape up to 120°C because the glass transition temperature of SU-8 increases with the soft-bake temperature (*20*). However, the microlens patterns on an SU-8 droplet may disappear with an insufficient prebake. In particular, the release of the SU-8 replica needs to be carried out at room temperature; otherwise, the gel-like SU-8 may not completely release from the PDMS mold. Next, a partially coherent UV light source from a photolithographic tool (Q4000 MA, Quintel Corporation, Morgan Hill, CA; 12 mW/cm$^2$ at 365 nm) was used to form a polymer cone and a waveguide under each microlens. The spherical arrangement of artificial ommatidia was determined by the spherical illumination of UV light, which can be achieved with a spherical mirror or a high numerical aperture (*NA*) condenser lens. In the experiment, an aspheric condenser lens (lens diameter of 23 mm, *F*/0.5, and back focal length of 6.9 mm) was chosen for ease of use in the experiment even though the angular span was limited by the *NA* of the condenser lens (Fig. 3H). For instance, the illumination angle for *F*/0.3 is ±45°. However, a spherical mirror-assisted illumination is recommended for UV illumination with a wide angle. The spherically UV-exposed SU-8 replica was then postexposure baked (at 90°C for 15 min) for photocross-linking and finally hard baked (at 150°C for 3 hours) for thermal cross-linking. Two scanning electron microscope (SEM) images showed that honeycomb-packed hexagonal microlenses of about 8370 (*F*/2.2, 25 μm in diagonal) are spherically arranged on a hemispherical polymer dome 2.5 mm in diameter (Fig. 3, I and J). Under the microlenses, self-aligned polymer cones and waveguide cores as well as cladding were observed by a cross-sectional SEM image (Fig. 3K).

Light from point light sources at infinity was coupled into the omnidirectionally arranged ommatidia with different coupling efficiency because each ommatidium covered a different direction. Consequently, the angular sensitivity function (ASF) of a single ommatidium can be reconstructed by measuring the relative intensity of the light at the distal end of each ommatidium, as proposed in a previous work (*21*). However, the actual measurement had not been carried out yet. The ASF of a single ommatidium in an artificial compound eye was measured by performing 3D optical sectioning based on laser scanning confocal microscopy (Fig. 4A). The optical sectioning of the artificial compound eye was carried out under normally incident light at 532 nm with a transmission confocal microscope (Zeiss 510, Carl Zeiss MicroImaging, Incorporated, Thornwood, NY) (Fig. 4B). Starting from the apex of the artificial compound eye, the vertical scanning was performed

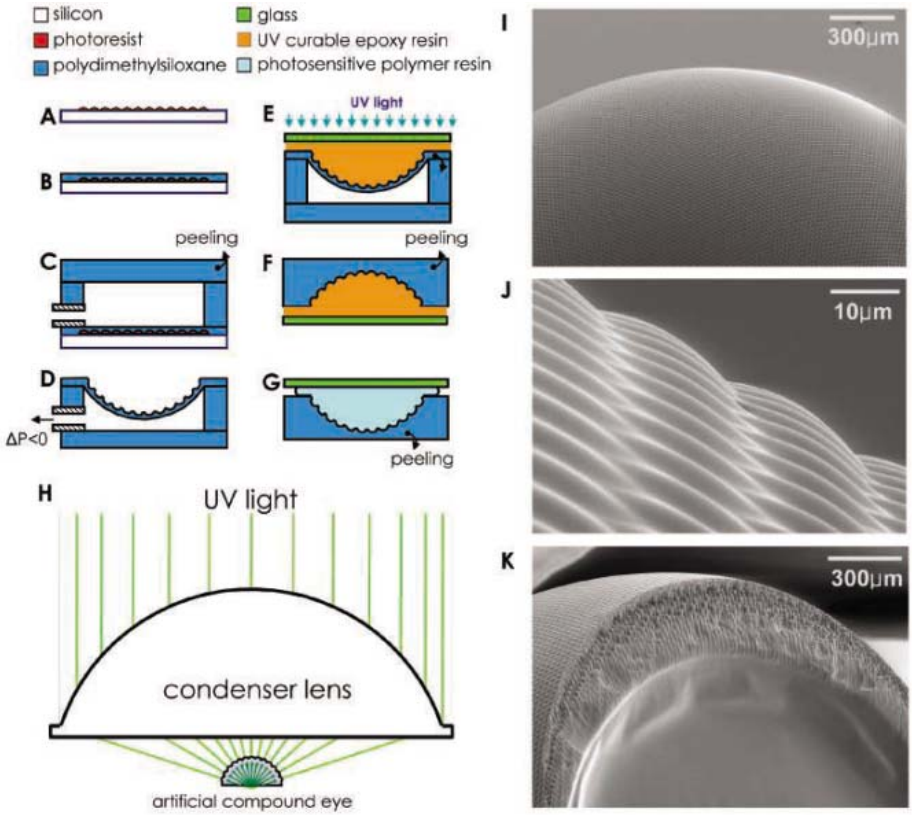


**Fig. 3.** The 3D polymer synthesis of biomimetic artificial compound eyes using (**A**) a honeycomb-packed polymer microlens process, (**B** to **G**) a reconfigurable microtemplating polymer process, and (**H**) a self-written waveguide process in photosensitive polymer resin by lens-assisted radial UV exposure. (A) Microlens template by a resist reflow method, (B) first PDMS molding, (C) PDMS bonding, (D) PDMS membrane deformation, (E) replication with UV curable polymer resin, (F) second PDMS molding, (G) photosensitive polymer resin (SU-8) molding, and (H) lens-assisted radial UV exposure and thermal cross-linking for self-written waveguides. SEM images of an artificial compound eye. (**I**) Spherical arrangement of 8370 artificial ommatidia on a hemispherical polymer dome 2.5 mm in diameter, (**J**) hexagonal microlenses, and (**K**) a cross section with the spherical arrangement of artificial ommatidia consisting of microlenses, polymer cones, and waveguide arrays.

over a range of 200 μm with a 2-μm increment. At each vertical increment, a 765-μm–by–765-μm area perpendicular to the incident light was laterally scanned with a 0.8-μm resolution. The confocal image on the *xy* plane was taken at 80 μm below the apex of the artificial compound eye. The cross-sectional confocal images scanned along the lines *aa′* and *bb′* are also shown at the top and right sides of the main image, respectively. The distributions of the relative output intensity measured along the two lines at the vertical position are also included on the bottom and left sides, respectively. The relative intensity of each peak represents the sensitivity of an individual ommatidium to different incidence angles. The observed distributions of relative intensity in *x* and *y* directions are slightly asymmetric because of the honeycomb packing of hexagonal microlenses.

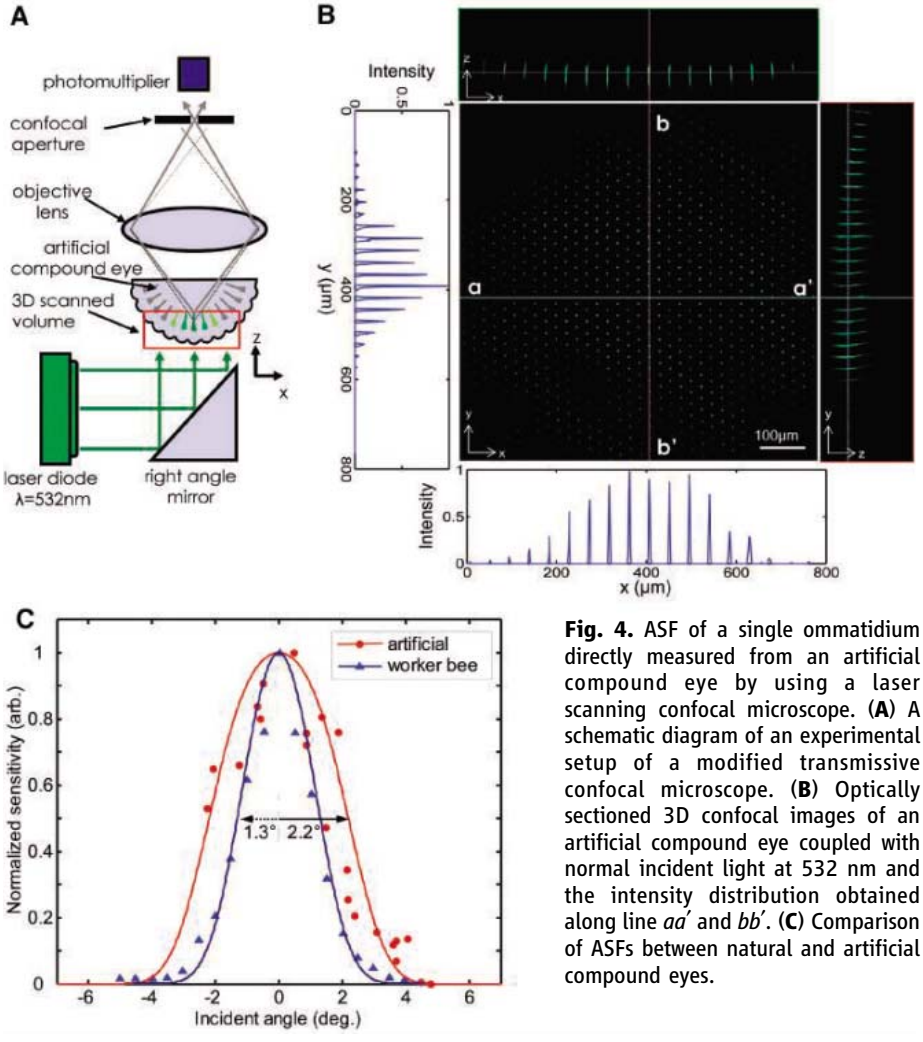


**Fig. 4.** ASF of a single ommatidium directly measured from an artificial compound eye by using a laser scanning confocal microscope. (**A**) A schematic diagram of an experimental setup of a modified transmissive confocal microscope. (**B**) Optically sectioned 3D confocal images of an artificial compound eye coupled with normal incident light at 532 nm and the intensity distribution obtained along line *aa′* and *bb′*. (**C**) Comparison of ASFs between natural and artificial compound eyes.

**Table 1.** Comparisons between artificial and natural compound eyes.

| | Artificial eyes | Natural eyes (bees) | Ref. |
|---|---|---|---|
| Shape of lens aperture | Hexagon | Hexagon | (*22*) |
| Maximal lens diameter | 25 μm | 20∼36 μm | (*22*) |
| *F* number | 1.8∼2.9 | 2.7∼3.3 | (*22*) |
| $N_F$ at 635 nm | 3.4∼5.5 | 2.4∼5.3 | |
| Number of lenses | 8370 | 3421∼4883 | (*22*) |
| Refractive index of lens | 1.584 | 1.363 | (*12*) |
| Index difference (core/cladding) | 0.029 (1.614/1.584) | 0.023 (1.363/1.340) | (*25*) |
| Waveguide shape | Cylindrical | Cylindrical | (*22*) |
| Waveguide core | 5.1∼6.3 μm | 2∼8 μm | (*22*) |
| Waveguide length | 150∼300 μm | 220∼350 μm | (*22*) |
| ASF | 1.1°∼4.4° | 1.6°∼4.7° | (*22*, *23*) |

To obtain the ASF of a single ommatidium, we first measured the orientation of each waveguide from the vertically scanned confocal images. The relative intensity distribution was plotted with respect to the incidence angle (Fig. 4C). Because of the tilting of the artificial ommatidium under confocal scanning, the measurement on the left-hand side was not complete. If a general symmetry is assumed, the acceptance angle, or the full width at half maximum of the measured ASF, is 4.4°. The value is comparable to those of natural compound eyes, which range from 1.6° to 4.7° (*22*). As shown in the superimposed curve, the acceptance angle of a worker bee ommatidium is ∼2.5° (*23*). We also reconstructed the theoretical ASFs by using the lens-waveguide coupling model proposed by Stavenga (*12*). The model takes both the diffraction by microscale lenses and the excitation of waveguide modes by the diffraction image into consideration. The results of reconstruction using only the fundamental waveguide mode were superimposed in Fig. 4C. We used the optical and structural parameters of the worker bee reported by Laughlin and Horridge (*23*) and Snyder and Pask (*24*). We point out that the use of only the fundamental mode of cylindrical waveguides for the reconstruction led to the best fit with experimental data for both cases. Although the approximation may be acceptable for waveguides in worker bee ommatidia, which support only two modes, it may not be applicable to the waveguides of artificial ommatidia, which support more than 10 modes. The unexpected agreement between the measured ASF and the single-mode approximated reconstruction suggests that the index distribution of the self-written waveguides deviates from the step profile and hence degrades the model. The current artificial ommatidium exhibits an acceptance angle wider than the interommatidial angle (∼1.5°), and it will suffer from overlap-induced image degradation. The main reason is that the curvature of the eyelet is increased by the large deformation of a polymer membrane during the polymer replication process. However, this problem can be resolved by controlling the local distribution of the microlenses. The optical sectioning technique not only enabled the visualization of the light propagation through microlenses but also facilitated the precise measurement of beam spot sizes at the focal plane of the microlenses and waveguide cores, waveguide modes, coupling loss, waveguide length, and most importantly the angular acceptance. More optical measurement results were comparable with the previously measured characteristics of the bee (Table 1). Our results show that both the physical dimensions and the optical characteristics of our artificial ommatidia are very comparable to those found in nature. Therefore, this 3D polymer fabrication method of biologically inspired optical systems has potential for a

broad range of optical applications, such as data storage and readout, medical diagnostics, surveillance imaging, and light-field photography.


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# A Population of Comets in the Main Asteroid Belt


**Henry H. Hsieh* and David Jewitt**



**Comets are icy bodies that sublimate and become active when close to the Sun. They are believed to originate in two cold reservoirs beyond the orbit of Neptune: the Kuiper Belt (equilibrium temperatures of ~40 kelvin) and the Oort Cloud (~10 kelvin). We present optical data showing the existence of a population of comets originating in a third reservoir: the main asteroid belt. The main-belt comets are unlike the Kuiper Belt and Oort Cloud comets in that they likely formed where they currently reside and may be collisionally activated. The existence of the main-belt comets lends new support to the idea that main-belt objects could be a major source of terrestrial water.**


Temperatures in the outer parts of the protoplanetary disk of the Sun, beyond a critical distance known as the snow line (*1*), were low enough for water to condense as ice. The icy planetesimals that formed beyond the snow line are the progenitors of today's comets—ice-rich bodies that sublimate when close to the Sun, producing distinctive unbound atmospheres ("comae") and tails (*2*). The active lifetimes [~$10^4$ years (*3*)] of comets that pass inside Jupiter's orbit are short relative to the age of the solar system ($4.6 \times 10^9$ years). This means that currently active comets must have only recently arrived in the inner solar system from cold reservoirs elsewhere, otherwise they would have exhausted their volatile material long ago. Two such originating reservoirs are well established. The Kuiper Belt (*4*) beyond Neptune (~30 to 50 AU from the Sun) supplies the so-called Jupiter-family comets (JFCs), whereas the much more distant Oort Cloud (*5*) (~3000 to 50,000 AU) supplies the Halley-family and long-period comets (*3*, *6*).

Although the dominant cometary reservoirs are located beyond the orbit of Neptune, the main cometary volatile, water, is stable as ice down to much smaller heliocentric distances (*7*), and it has long been suspected that other populations (such as the Hilda asteroids at 4 AU and the jovian Trojans at 5 AU) might be ice-rich, dormant comets (*8*, *9*). However, the active comet population we see today consists mainly of objects from the Kuiper Belt and Oort Cloud that have been scattered onto Jupiter-crossing orbits by gravitational interactions with the giant planets (*3*, *10*). Even the dynamically peculiar comet 2P/Encke is believed to have originated in the Kuiper Belt, albeit with an orbital evolutionary history strongly influenced by nongravitational forces induced by cometary outgassing (*11*, *12*).

Despite occupying a thoroughly asteroidal orbit in the main belt between the orbits of Mars and Jupiter, asteroid 7968 Elst-Pizarro (also known as comet 133P/Elst-Pizarro) was observed to eject dust like a comet when near perihelion in both 1996 and 2002 (*13*, *14*).

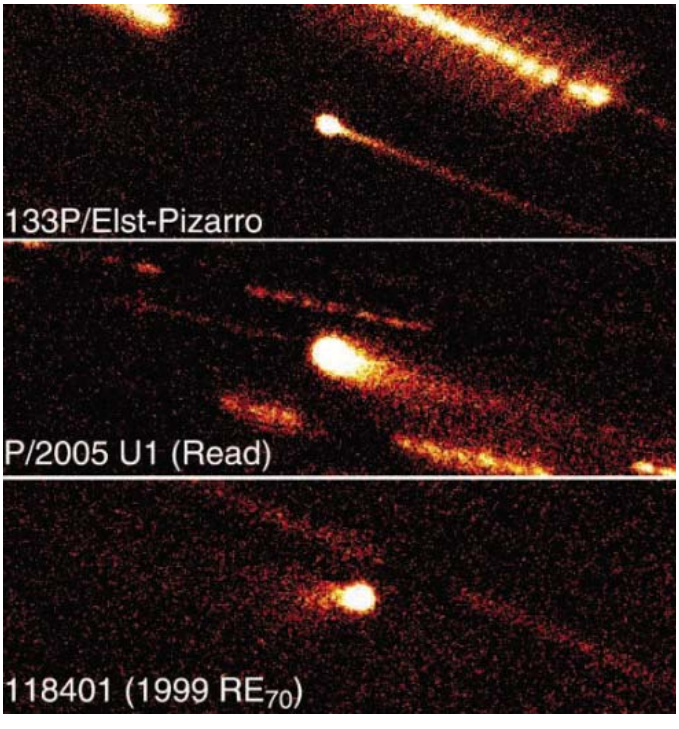


**Fig. 1.** *R*-band (wavelength 0.65 μm) images of MBCs 133P/Elst-Pizarro on 7 September 2002 (*14*), P/2005 U1 (Read) on 10 November 2005, and 118401 (1999 $RE_{70}$) on 27 December 2005 (all dates UT). All images are composites (0.5′ by 1.5′ in size, with north at the top and east to the left) from data taken at the University of Hawaii 2.2-m telescope on Mauna Kea, and represent 1.1 hours, 1.9 hours, and 2.8 hours of total effective exposure time, respectively. Streaked objects in each panel are background stars and galaxies that have been trailed by the nonsidereal motions of the comets. Geometric circumstances of these observations are given in Table 1.


Institute for Astronomy, University of Hawaii, 2680 Woodlawn Drive, Honolulu, HI 96822, USA.

*To whom correspondence should be addressed. E-mail: hsieh@ifa.hawaii.edu

Motivated by these observations, we have been conducting an optical survey of asteroids in the main belt in search of similar behavior. In conducting this survey, we have used both small-aperture (1 to 2 m) and large-aperture (8 to 10 m) telescopes in Hawaii, Chile, and Taiwan, and have observed ~300 small (kilometer-sized) main-belt asteroids over the past 3 years.

Recently, two more main-belt objects—P/2005 U1 (Read) (*15*), discovered serendipitously, and asteroid 118401 (1999 $RE_{70}$), found to be active by our survey using the 8-m Gemini telescope—have been found with comet-like morphologies (Fig. 1 and Table 1). Together, these three objects (Table 2) form a new class of comets having stable orbits completely confined to the main asteroid belt: the main-belt comets (MBCs).

The image data (Fig. 1) leave no doubt that these asteroids are ejecting dust and so satisfy the observational definition of comets (*10*). Dust ejection velocities in the two most recently discovered MBCs were estimated from the sunward extents of their comae. The sunward extent of a coma is proportional to the square of the ejection velocity divided by the acceleration due to radiation pressure. Velocities estimated for the MBCs are ~100 m $s^{-1}$, greatly in excess of the velocities that would be expected for electrostatic levitation [~1 m $s^{-1}$ (*16*)] or rotational ejection (on the order of the escape velocity, or ~1 m $s^{-1}$ for an object of radius ~1 km). Additionally, the activity of all three MBCs is observed to persist for several weeks or months, much longer than would be expected for impact-generated dust ejection but consistent with sublimation-driven dust ejection (*14*). In the case of Elst-Pizarro, activity is seen to recur over a finite portion of its orbit close to perihelion; this observation is again consistent with a sublimation-driven origin (*14*). Physically, the MBCs are bona fide comets. Dynamically, however, all three are completely asteroidal in character (Fig. 2), with orbits unlike those of any other known comets.

Could the MBCs be comets from the Kuiper Belt or Oort Cloud that have become trapped in asteroid-like orbits? Published dynamical simulations suggest that this is not the case, because they cannot reproduce the transfer of comets to main-belt orbits (*11*). Likewise, it is extremely unlikely that the MBCs could have resulted from collisional deflection of comets passing through the main belt, for two reasons: (i) the long time scales of such collisions (at least $10^7$ times the dynamical lifetimes of the JFCs), and (ii) the implausibility of such collisions delivering fragments precisely onto nearly circular, low-inclination main-belt orbits, given the much more eccentric and more inclined orbits of the presumed parent comets. Unless nongravitational forces associated with asymmetric cometary outgassing can somehow be invoked to solve this problem, an origin for the MBCs in the Kuiper Belt or Oort Cloud appears improbable.

It is more likely that the MBCs are intrinsically icy bodies, formed and stored at their current locations, that have been activated by some recent trigger. A recent trigger is required because exposed, dirty water ice located at the subsolar point of an MBC at a heliocentric distance of 2.4 to 2.9 AU (Table 1) will sublimate and recede at a rate on the order of 1 m per year. Given the kilometer-scale sizes (Table 2) of the currently known MBCs, the active lifetimes of these objects once sublimation begins must be considerably shorter than 1000 years. Models show, however, that buried ice at these distances could be protected against sublimation over the entire age of the solar system by even a relatively thin (~1 to 100 m) layer of surface regolith (*17*). Sublimation could then be triggered by a collision able to penetrate this insulating inactive layer, exposing deeply buried ice to the heat of the Sun. The behavior of Elst-Pizarro suggests that the resulting activity might then continue for several months before subsiding, with recurring outbursts continuing on a seasonal basis for several more years (*14*).

The discovery of the MBC class is scientifically interesting on several levels. Geochemical and spectroscopic evidence for hydrated minerals on main-belt asteroids is best explained if those asteroids were once bathed in liquid water (*18*, *19*). The absence of hydration features in certain asteroids has been interpreted as a sign that their ice was never heated to the liquid phase and presumably remains frozen inside (*20*). The MBCs are optically faint and have not been spectroscopically studied to see whether they show hydration features. However, two-thirds of the asteroids in the region where the MBCs are found (~3 AU) show no evidence for hydration (*21*) and thus are candidates for containing water as ice.

Dynamically, two of the three MBCs (Elst-Pizarro and 118401) are associated with the Themis collisional family, with P/Read falling just outside this family because of its slightly high eccentricity. This family association may be simply an artifact of observational selection because our survey has been focused on Themis family objects, although a number of other main-belt asteroids were also observed. More observations are needed to determine the true distribution of MBCs; it is possible that non-Themis objects that outgas also exist but have so far escaped detection because they were not emphasized in our initial survey sample. The velocity dispersion among the known MBCs is ~1 km $s^{-1}$, which is much larger than typical velocity dispersions among fragments of split comets (~1 m $s^{-1}$) but is comparable to the velocity dispersion expected from a collisionally shattered body. The collisional lifetime of a 5-km body in the main belt is

**Table 1.** Observational circumstances of MBCs during observed periods of activity. Position data shown are from JPL's online ephemeris generator and include heliocentric distance $R$, geocentric distance $\Delta$, and phase angle $\alpha$ (Sun-object-Earth). For new observations of P/Read and 118401, we also report approximate mean $R$-band magnitudes ($m_R$) measured at the times of observation. ESO, European Southern Observatory; UH, University of Hawaii.

| Object | UT date | Telescope | $R$ (AU) | $\Delta$ (AU) | $\alpha$ (°) | $m_R$ |
|---|---|---|---|---|---|---|
| 133P/Elst-Pizarro | 14 Jul 1996 | ESO 1.0 m (*13*) | 2.65 | 1.77 | 13.1 | 18.3 |
| | 19 Aug 2002 | UH 2.2 m (*14*) | 2.86 | 2.05 | 14.5 | 20.10 ± 0.10 |
| | 07 Sep 2002 | UH 2.2 m (*14*) | 2.89 | 1.94 | 8.2 | 19.70 ± 0.05 |
| P/2005 U1 (Read) | 24 Oct 2005 | Spacewatch 0.9 m (*15*) | 2.42 | 1.46 | 8.7 | 20.2 |
| | 10 Nov 2005 | UH 2.2 m | 2.44 | 1.45 | 0.6 | 19.28 ± 0.05 |
| 118401 (1999 $RE_{70}$) | 26 Nov 2005 | Gemini 8 m | 2.59 | 1.82 | 16.4 | 19.16 ± 0.05 |
| | 27 Dec 2005 | UH 2.2 m | 2.60 | 2.19 | 21.5 | 19.60 ± 0.05 |

**Table 2.** Orbital and physical parameters of MBCs. Orbital data are from JPL's online database and include semimajor axis $a$, eccentricity $e$, inclination $i$, Tisserand parameter $T_J$, perihelion distance $q$, and aphelion distance $Q$. The Tisserand parameter is an approximately constant dynamical quantity that reflects the degree of an object's dynamical coupling with Jupiter and is commonly used to classify orbits as cometary or asteroidal. Most comets have $T_J < 3$; most asteroids have $T_J > 3$ (*28*, *29*). We also report approximate effective diameters $d_e$ for P/Read and 118401 estimated from apparent $R$-band magnitudes (Table 1) and an assumed geometric albedo of 0.04. The estimated contribution to object brightness due to coma (as determined from comparison of MBC surface brightness profiles to field star profiles) has been subtracted.

| Object | $a$ (AU) | $e$ | $i$ (°) | $T_J$ | $q$ (AU) | $Q$ (AU) | $d_e$ (km) |
|---|---|---|---|---|---|---|---|
| 133P/Elst-Pizarro | 3.156 | 0.165 | 1.39 | 3.184 | 2.636 | 3.677 | 5.0 (*14*) |
| P/2005 U1 (Read) | 3.165 | 0.253 | 1.27 | 3.153 | 2.365 | 3.965 | 2.2 |
| 118401 (1999 $RE_{70}$) | 3.196 | 0.192 | 0.24 | 3.166 | 2.581 | 3.811 | 4.4 |

roughly $10^9$ years (*22*). Thus, although it is possible that the MBCs are collisionally produced fragments of precursor asteroids, a recent disruptive collision is unlikely, and—given that observational selection effects can just as easily explain these objects' orbital similarity—we do not believe that the MBCs necessarily originated from a common parent.

The similarity of the semimajor axes of the MBCs (Table 2) may be important in other respects. Thermal averaging inside the nuclei will lead to deep interior temperatures near the local blackbody value. At 3.2 AU (the approximate semimajor axis of all three MBCs), this temperature is 155 K, at which ice is thermodynamically stable. At smaller distances, higher deep interior temperatures may prevent the survival of ice over the age of the solar system. Careful observations in search of comae on closer asteroids are needed. Present-day surface water ice and even possible water sublimation have been reported on 1 Ceres (*23*, *24*), although no visible cometary activity has ever been observed, as well as on Elst-Pizarro (*14*), the prototype MBC. Our observations show that the snow line was once within the asteroid belt and suggest that buried water ice in the main belt may be common.

The outer main belt has been proposed as a likely source of terrestrial water (*25*). In this regard, it would be valuable to determine the isotopic composition (especially D/H) in ice from the MBCs for comparison with the isotopic composition of the oceans. Given their proximity to Earth, the MBCs make attractive targets for spacecraft sampling missions having this scientific objective. In addition, the noble gases on Earth and the terrestrial planets may have been delivered by the impact of asteroids and comets (*26*). High-temperature ice from the main belt is a possible carrier of at least the less volatile noble gases and, again, in situ measurements would place useful constraints on the magnitude of a possible main-belt source.

Finally, the MBC class was identified from limited observational data. In our survey, we discovered one new MBC (asteroid 118401) from observations of ~300 main-belt objects (including ~150 Themis-family asteroids). Scaling to the currently known population of >50,000 small asteroids (with radii of <10 km) in the outer main belt beyond 3 AU (with ~2000 in the Themis-family region), we estimate that there could be 15 to 150 currently active MBCs. We caution, however, that our survey was designed to maximize the chances of finding new Elst-Pizarro–like objects, not to provide a statistically significant representation of the main-belt population. Furthermore, the number of dormant MBCs (i.e., icy asteroids that have not yet been collisionally activated) must certainly be larger than the number of currently active MBCs. More MBCs may soon be identified by synoptic all-sky survey telescopes currently under development, such as the Panoramic Survey Telescope and Rapid Response System (Pan-STARRS) (*27*).

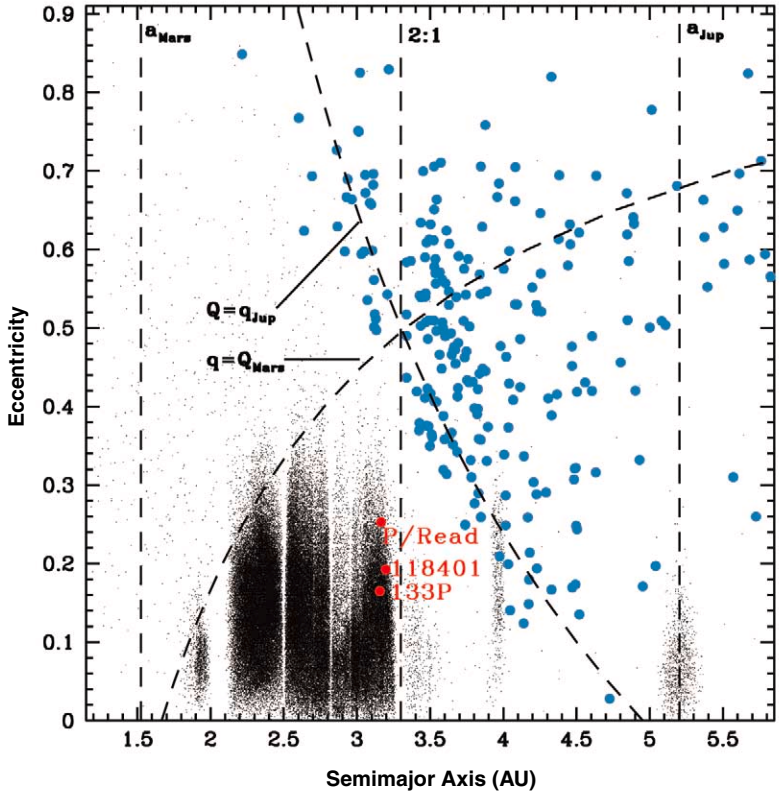


**Fig. 2.** Plot of semimajor axis versus eccentricity for all numbered asteroids (small black dots) and comets (large blue dots) tabulated by JPL as of 14 December 2005. MBCs 133P/Elst-Pizarro, P/2005 U1 (Read), and 118401 (1999 $RE_{70}$) are plotted in red. Vertical dashed lines mark the semimajor axes of Mars and Jupiter ($a_{Mars}$, $a_{Jup}$) and the 2:1 mean-motion resonance with Jupiter (commonly considered the outer bound of the classical main belt), as labeled. Curved dashed lines show the loci of orbits with perihelia equal to Mars' aphelion ($q = Q_{Mars}$) and orbits with aphelia equal to Jupiter's perihelion ($Q = q_{Jup}$). Objects plotted above the $q > Q_{Mars}$ line are Mars-crossers; objects plotted to the right of the $Q < q_{Jup}$ line are Jupiter-crossers. The MBCs, like the majority of main-belt asteroids, approach neither Mars nor Jupiter.

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# Iron-Rich Post-Perovskite and the Origin of Ultralow-Velocity Zones

Wendy L. Mao,[1]* Ho-kwang Mao,[2,3] Wolfgang Sturhahn,[4] Jiyong Zhao,[4] Vitali B. Prakapenka,[5] Yue Meng,[3] Jinfu Shu,[2] Yingwei Fei,[2] Russell J. Hemley[2]

The boundary layer between the crystalline silicate lower mantle and the liquid iron core contains regions with ultralow seismic velocities. Such low compressional and shear wave velocities and high Poisson's ratio are also observed experimentally in post-perovskite silicate phase containing up to 40 mol% $FeSiO_3$ endmember. The iron-rich post-perovskite silicate is stable at the pressure-temperature and chemical environment of the core-mantle boundary and can be formed by core-mantle reaction. Mantle dynamics may lead to further accumulation of this material into the ultralow-velocity patches that are observable by seismology.

Earth's core-mantle boundary (CMB) exhibits a complex seismic signature. One of the most puzzling features is the presence of 5- to 40-km-thick, ultralow-velocity zones (ULVZ) (*1*–*3*) in which the compressional wave ($V_P$) and shear wave ($V_S$) velocities are depressed by 5 to 10% and 10 to 30%, respectively, relative to the Preliminary Earth Reference Model (PREM), and the Poisson's ratio (ν) is increased from 0.3 to 0.4 (*4*, *5*) (Table 1). There are also seismic observations of considerably increased density (up to 50%) in some ULVZ (*6*). Thermal effects or phase transitions alone may not be sufficient to explain such large reductions of velocities; partial melting (*7*, *8*) or chemical enrichment of heavy elements such as Fe may be necessary (*9*). The unusually high Poisson's ratio (ν) in ULVZ has often been interpreted as evidence for partial melting. No Fe-rich silicate was previously known to exist above 25 GPa where Fe-rich ringwoodite disproportionates into mixed oxides (*10*), and the maximum Fe content in mantle ferromagnesian silicates with the perovskite structure was thought to be $x = \mathrm{Fe}/(\mathrm{Fe} + \mathrm{Mg}) < 0.15$ (*11*).

The identification of post-perovskite (denoted ppv) transition in $MgSiO_3$ (*12*) brought a new paradigm for the D″ zone (*1*). Although the isochemical perovskite-to-ppv transition itself only increases the seismic velocity, the ppv phase can retain a large amount of Fe leading to dramatically increased density (*13*), thus providing a new, alternative candidate. Hence, we use high-pressure nuclear resonant inelastic x-ray scattering (NRIXS) spectroscopy and x-ray diffraction (XRD) to demonstrate that the Fe-rich silicate indeed has the low-velocity, high-ν signature of the ULVZ. Synchrotron NRIXS spectroscopy allows determination of the phonon density of state (DOS) related to $^{57}$Fe isotope in the sample. With the assumption of evenly distributed $^{57}$Fe in a single, Fe-bearing phase, the low-energy portion of the phonon DOS yields the Debye sound velocity ($V_D$) of the phase, which is related to $V_P$ and $V_S$ by

$$\frac{3}{V_D{}^3} = \frac{1}{V_P{}^3} + \frac{2}{V_S{}^3} \qquad (1)$$

In addition, $V_P$ and $V_S$ are related to seismic bulk sound speed ($V_\phi$), bulk modulus ($K$), and density (ρ) by

$$V_\phi{}^2 = V_P{}^2 - (4/3)V_S{}^2 = K/\rho \qquad (2)$$

where $K$ and ρ are parameters in pressure-volume equation of state which can be obtained from high-pressure XRD. $V_P$ and $V_S$ can thus be solved from Eqs. (1) and (2), and ν can be obtained from the known $V_P$ and $V_S$ as follows:

$$\nu = \frac{\left(\frac{V_P}{V_S}\right)^2 - 2}{2\left[\left(\frac{V_P}{V_S}\right)^2 - 1\right]} \qquad (3)$$

We studied $Fe_{0.4}Mg_{0.6}SiO_3$ (Fs40) up to 170 GPa using synchrotron XRD at beamlines 13-IDD and 16-IDB of the Advanced Photon Source (APS) (*14*). We compressed Fs40 orthopyroxene starting materials to 130 GPa and laser-heated to 2000 K, which converted the sample into very well crystallized single-phase ppv, which shows sharp diffraction peaks (Fig. 1) and approximately 50% more peaks than previous reports (*12*, *13*). The pressure was first increased incrementally to nearly 170 GPa and then decreased down to 125 GPa. At each pressure-increasing or -decreasing step, the sample was laser annealed to 2000 K to release possible strain and confirm ppv stability. XRD patterns were collected after temperature quenching at each pressure step and used for calculation of the lattice parameters, unit cell volume, and equation of state for Fs40 ppv

**Table 1.** Seismic wave velocities above ULVZ and in ULVZ in comparison to the present Fs40 ppv results.

| | $V_P$, km/s | $V_S$, km/s | $\nu$ |
|---|---|---|---|
| PREM, mantle side of CMB (*5*) | 13.72 | 7.26 | 0.30 |
| ULVZ (*4*) | 12.35 | 5.08 | 0.40 |
| Fs40 ppv at 130 GPa-300 K | 12.72 | 4.86 | 0.41 |
| Fs40 ppv at 130 GPa-3000 K | 11.91 | 4.05 | 0.41 |

[1]Lujan Neutron Scattering Center, Los Alamos National Laboratory, Los Alamos, NM 87545, USA. [2]Geophysical Laboratory, Carnegie Institution of Washington, Washington, DC 20015, USA. [3]High Pressure Collaborative Access Team, [4]X-ray Operations and Research, Advanced Photon Source, Argonne National Laboratory, Argonne, IL 60439, USA. [5]University of Chicago, Chicago, IL 60637, USA.

*To whom correspondence should be addressed. E-mail: wmao@lanl.gov

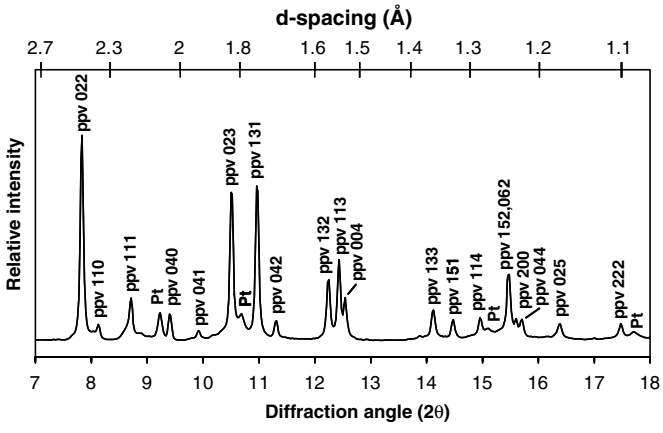


**Fig. 1.** X-ray diffraction pattern for Fs40 ppv at 138 GPa (λ = 0.3344 Å).

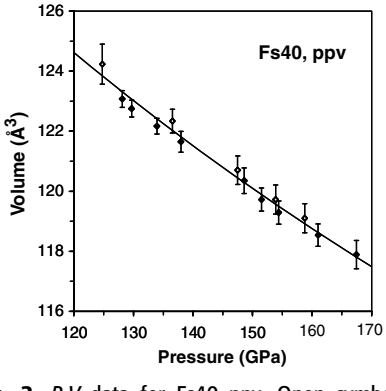


**Fig. 2.** *P-V* data for Fs40 ppv. Open symbols, increasing pressure; closed symbols, releasing pressure; solid line, third-order Birch-Murnaghan EOS fit to the measurements.

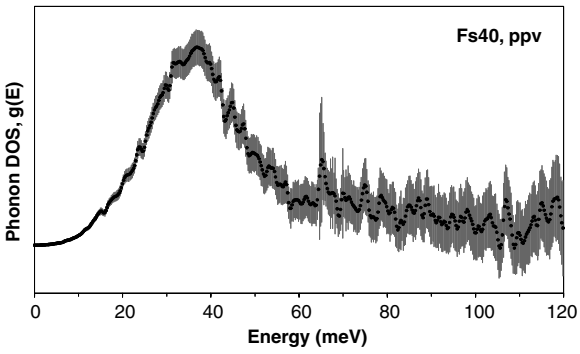


**Fig. 3.** NRIXS spectra showing the phonon DOS for ppv phase of Fs40 at 130 GPa after temperature quench from 2000 K.

(Fig. 2). We found that ρ = 6.08 g/cm$^3$, $K$ = 792 GPa, and $V_\Phi$ = 11.43 km/sec at 130 GPa.

For the NRIXS experiment (*14*), we used a panoramic diamond-anvil cell to synthesize the Fs40 ppv sample at 130 GPa and 2000 K. After confirming the formation of ppv by x-ray diffraction at beamline 16-IDB, we carried out the NRIXS experiment at ambient temperature at beamline 3-ID of the APS. NRIXS spectra were collected in situ and converted to give the partial (Fe-related) DOS (*15*) (Fig. 3). The Debye sound velocity ($V_D$) was found to be 5.51 ± 0.03 km/sec from a fit to the low energy (long wavelength) portion of the DOS to a parabolic function (*16*). Using Eqs. 2 and 3 and our $V_\Phi$ and $V_D$ values, we determined $V_P$ = 12.72 ± 0.12 km/s, $V_S$ = 4.86 ± 0.03 km/s, and ν = 0.41 ± 0.01 for Fs40 ppv at 130 GPa and 300 K. These parameters are close to those in the ULVZ at high temperature (Table 1).

Based on a first-order approximation of $\partial V/\partial T$ = –0.0003 km/s-K (*17*), temperature correction to the CMB conditions will reduce the velocities and increase the Poisson's ratio of Fs40 ppv beyond the ULVZ values (Table 1). Lower $FeSiO_3$ content than 40% or additional solid phases such as magnesiowüstite will bring the values into agreement with those observed in ULVZ. The present results indicate that the addition of Fe is sufficient for explaining seismic features of ULVZ, thus providing an alternative explanation to partial melting.

At the CMB, the silicate is in contact with the liquid Fe alloy. A 10- to 100-m-thick, reaction veneer of the Fe-rich ppv silicate could form at the interface through static, diffusive process alone. However, the CMB can hardly be regarded as static. We can expect turbulence, shear-induced dilation (*18*), and infiltration to promote local reactions to the kilometer levels. The Fe-rich ppv would be too heavy to rise in the mantle and would pile up beneath upwelling areas to form seismically observable ULVZ patches that could correlate with active hot spots and upwelling areas (*19*–*21*). With such a cumulative mechanism, we could also expect relics of ULVZ that do not correlate with the present day upwelling [for examples, see (*4*, *22*)] but reveal geodynamic patterns in Earth's history.

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### Supporting Online Material

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Materials and Methods
References

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# Chronology for the Aegean Late Bronze Age 1700–1400 B.C.

**Sturt W. Manning,[1,2]* Christopher Bronk Ramsey,[3] Walter Kutschera,[4] Thomas Higham,[3] Bernd Kromer,[5] Peter Steier,[4] Eva M. Wild[4]**

**Radiocarbon (carbon-14) data from the Aegean Bronze Age 1700–1400 B.C. show that the Santorini (Thera) eruption must have occurred in the late 17th century B.C. By using carbon-14 dates from the surrounding region, cultural phases, and Bayesian statistical analysis, we established a chronology for the initial Aegean Late Bronze Age cultural phases (Late Minoan IA, IB, and II). This chronology contrasts with conventional archaeological dates and cultural synthesis: stretching out the Late Minoan IA, IB, and II phases by ~100 years and requiring reassessment of standard interpretations of associations between the Egyptian and Near Eastern historical dates and phases and those in the Aegean and Cyprus in the mid–second millennium B.C.**

The second millennium B.C. saw several major civilizations develop in the Aegean (Greece, Crete, and Anatolia) and on Cyprus, which became integrated into the trading and cultural worlds of the ancient Near East and Egypt. The analysis of these civilizations and their relationships depends upon an accurate chronology that establishes linkages and developmental frameworks. Chronologies for Aegean and east Mediterranean cultures during the second millennium B.C. have usually been derived from comparisons of artifact and style associations with those in the Near East, which can be related to the approximate historical chronologies of Egypt or Mesopotamia (*1*, *2*). Where there were extensive cultural exchanges, this approach seems sound, particularly during the Amarna period in the mid–14th century B.C. and again, but a little less clearly, during the Middle Kingdom period in the 19th to 18th centuries B.C. (*2*). But chronologies for other periods, including the initial Late Bronze Age, are ambiguous. This time marked the acme of New Palace civilization on Crete, the Shaft Grave period on mainland Greece, and the development of major new coastal polities on Cyprus.

Existing carbon-14 ($^{14}$C) dates for materials linked to the earlier Late Bronze Age cultural phases on Crete (Late Minoan IA, IB, and II, which are abbreviated as LMIA, LMIB, and LMII, respectively) or the associated Aegean region generally indicate ages older than ex-

pected. One critical tie point is the age of the Santorini eruption, which distributed tephra widely across the region. This event is placed in the mature or late LMIA phase and has conventionally been dated ~1525–1500 B.C. (*1*–*6*), but for 30 years $^{14}$C dates have yielded earlier ages around 100 years older, leading to controversy (*7*–*15*).

We identify four key areas as central to resolving the current debate: (i) provision of robust, consistent, high-quality $^{14}$C data by more than one laboratory; (ii) calibration of the $^{14}$C evidence with the latest high-precision data sets, but also tests to show that outcomes are robust enough not to be sensitive to small changes in the calibration curve; (iii) consideration of whether volcanic $CO_2$ emissions may have affected $^{14}$C ages obtained from Santorini; and (iv) appropriate, holistic analysis integrating $^{14}$C data, archaeological information, and the $^{14}$C calibration curve.

Here, we report a $^{14}$C chronology for the Aegean at the beginning of the Late Bronze Age and for the wider region in the mid–second millennium B.C. (*16*). We focused on short-lived samples, which should offer ages contemporary with their use, obtained sets of ages from the successive stratigraphic phases from LMIA through LMII, and used multiple-set simultaneous calibration [using Bayesian modeling (*17*, *18*)] to resolve single-case dating ambiguities caused by the irregular shape of the $^{14}$C calibration curve. Such a time-series comparison to the $^{14}$C calibration curve also controls against any significant contamination (e.g., by volcanic $CO_2$), because affected data should not offer a good fit.

We sampled sites in the southern Aegean region (fig. S1), including Santorini, from the LMIA, LMIB, and LMII phases to obtain 100 $^{14}$C dates (table S1) and selected for contexts where a minimum of two dates could be obtained (to try to obtain some control on reproducibility and outliers). We also included 27 previously published high-quality dates from the same or very similar samples (*19*–*21*) (table S1). Dates were analyzed by using the IntCal04 $^{14}$C calibration curve (*22*). We studied the robustness of the conclusions by varying the data sets, the stratigraphic model, and the calibration curve itself [with use of IntCal98 (*23*)].

To establish data quality, we divided 17 of our Aegean Late Bronze Age samples, either identical tree-ring fractions or groups of same species seeds from the identical prehistoric storage container, between the Oxford and Vienna laboratories. In addition, one prehistoric wood-charcoal sample and one more recent known-age tree-ring sample were divided between Oxford and Heidelberg and between Vienna and Heidelberg, respectively. The Oxford-Vienna data, 23 measurements from Oxford (where six samples were in fact measured twice, independently, on two different accelerators) and 17 measurements from Vienna, corresponded well, with a mean difference of only 11.7 $^{14}$C years (Fig. 1). Just 2 of the 17 pairs offered divergent outcomes at the 95% confidence level using a $\chi^2$ test [sample key 7, where $T = 5.6 > 3.8$, and sample key 15, where $T = 22.5 > 3.8$ (*16*)]; the sample key 15 case offered the only clear disagreement between the laboratories: This sample is an irrelevant early *terminus post quem* date, so its inclusion or exclusion makes no difference to our analyses below. The Oxford-Heidelberg pair returned almost identical data. The five Vienna measurements on the same tree-ring decade show a tight scatter of results with the mean close to the Heidelberg high-precision estimate, and all the constituent data include the known dendro-age within their 1σ (68.2% confidence) calibrated calendar age ranges (fig. S6). These comparisons support the accuracy of our Aegean $^{14}$C measurements.

Our measurements include 13 different short-lived samples (groups of seeds) from four larger seed samples recovered in situ from prehistoric storage containers found in the volcanic destruction level (VDL) on Santorini (samples shown as 16 to 19 in Fig. 1 from Akrotiri, Thera). An issue sometimes raised with regard to $^{14}$C measurements from the final VDL on Santorini is whether volcanic $CO_2$ might be affecting the samples and producing ages that are too old (*15*). Such volcanic effects, when observed (*24*, *25*), are typically only relevant either close to a vent or in low-lying areas or sinks. It is possible that some of the samples found at Akrotiri on Thera could have been so affected, although none from secure VDL contexts exhibit the large old-age offsets typical of such contaminated samples. However, it would seem unlikely that all the VDL samples from different pots and different crops were consistently affected. Our data, and other published Santorini VDL data available as the result of measurements on full seeds, groups of seeds, or a short-lived twig (*19*–*21*), $n = 28$, show a consistent age of 3344.9 ± 7.5 $^{14}$C years before the present [$^{14}$C yr B.P. from A.D. 1950 (*23*)], which equates to 1683–1611 B.C. at 2σ confidence with the use of IntCal04 (*22*) (Fig. 2B). Samples from Miletos (western Turkey) and Trianda (Rhodes) yield ages compatible with those from LMIA on Santorini (Fig. 2A and table S1). As a further test, we modeled the Santorini VDL age range excluding all data from Santorini, avoiding any possible volcanic effect. This placed the VDL at 1668–1585 B.C.

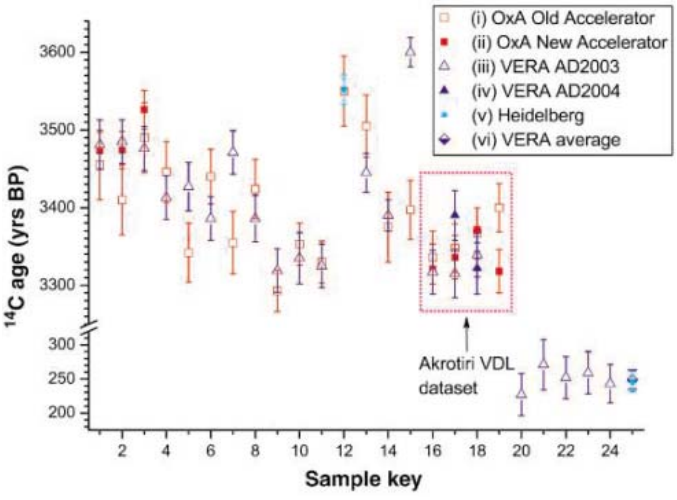


**Fig. 1.** Comparison of $^{14}$C age estimates for fractions of identical Aegean samples between (i) Oxford Old Accelerator (samples measured from A.D. 2000–2002), (ii) Oxford New Accelerator (measured in A.D. 2003), (iii) VERA (measured in A.D. 2003), (iv) VERA (measured in 2004), and (v) Heidelberg. The weighted average of the five VERA measurements on a sample of known age wood are shown (vi) as compared to the Heidelberg measurement of the same sample. Sample key includes the following samples: 1, Trianda AE1024 rings 21 to 30; 2, Trianda AE1024 rings 11 to 20; 3, Trianda AE1024 rings 1 to 10; 4, Akrotiri M4N003 rings 6 to 8; 5, Akrotiri M4N003 rings 3 to 5; 6, Akrotiri M4N003 rings 7 and 8; 7, Akrotiri M4N003 rings 5 and 6; 8, Akrotiri M4N003 rings 3 and 4; 9, Akrotiri 65/N001/I2 ring 3; 10, Akrotiri 65/N001/I2 ring 2; 11, Akrotiri 65/N001/I2 ring 1; 12, Akrotiri M54/2/VII/60/SE>247; 13, Kommos K85A/62D/9:92; 14, Kommos K85A/66B/4:22+23; 15, Kommos K85A/62D/8:83; 16, Akrotiri M31/43 N047; 17, Akrotiri M2/76 N003; 18, Akrotiri M7/68A N004; 19, Akrotiri M10/23A N012; 20 to 24, Çatacık tree rings A.D. 1640 to 1649; and 25, weighted average VERA Laboratory data (samples 20 to 24) versus Heidelberg measurement of same sample. Samples 20 to 25 also offer a known-age test. All five VERA $^{14}$C measurements included the correct calendar age range within their 1σ calibrated ranges (*17*, *22*), as does the VERA weighted average and the high-precision Heidelberg measurement. Error bars indicate 1σ ranges.

[1]Department of Classics, Cornell University, 120 Goldwin Smith Hall, Ithaca, NY 14853–3201, USA. [2]Department of Archaeology, School of Human and Environmental Sciences, University of Reading, Reading RG6 6AB, UK. [3]Oxford Radiocarbon Accelerator Unit, Research Laboratory for Archaeology and the History of Art, Oxford University, Dyson Perrins Building, South Parks Road, Oxford OX1 3QY, UK. [4]Vienna Environmental Research Accelerator (VERA) Laboratory, Institut für Isotopenforschung und Kernphysik, Universität Wien, Währinger Straße 17, A-1090 Wien, Austria. [5]Heidelberger Akademie der Wissenschaften, Institut für Umweltphysik der Universität Heidelberg, Im Neuenheimer Feld 229, D-69120 Heidelberg, Germany.

*To whom correspondence should be addressed. E-mail: sm456@cornell.edu

(2σ) and 1659–1624 B.C. (1σ) (Table 1 and fig. S5), consistent with results including the Santorini data (Fig. 2 and Table 1).

We constructed Bayesian models for the analysis of the sets of LMIA to II age determinations. The models account for the stratigraphic order of the samples implying a definite chronological sequence and include variable parameters like boundary dates of phases. The analysis calculates how successfully the $^{14}C$ measurements conform to the prior knowledge of the chronological sequence and yields estimates for parameters and narrowed (constrained) dates for the $^{14}C$ samples (*16*). In model 1, we represent the archaeological data as simply reduced to the secure evidence, with the criteria being two or more data comprising each specific grouping or sample set (Fig. 2A). No interpretative development has occurred in this model. We considered other more refined models or variations (*16*), and the outcomes were similar in all cases (figs. S2 to S5 and table S3).

The sequence model (Fig. 2A) offers a coherent chronology from LMIA through LMII. This model includes two tree-ring samples where defined sequence (D_Sequence) analysis (so-called wiggle matching, WM, where the time differences of the elements within the sequence are exactly known) was possible; these samples help set *terminus post quem* (tpq) ranges for the late Middle Bronze Age and/or early LMIA phase, respectively, and for the specific Akrotiri VDL (*16*). The internal consistency of the Aegean archaeological sequence and our data over the three centuries compared to the Northern Hemisphere atmospheric $^{14}C$ record indicates that no unusual offset exists within the Aegean sequence.

All of the ages calculated for the transitions or the events or phases for the LMIA and LMIB phases using OxCal (*17*) and the new internationally recommended IntCal04 $^{14}C$ calibration data set (*22*), or the previous IntCal98 data set (*23*), are significantly earlier than many previous estimates (*1–7*, *11*, *12*, *14*, *15*) (Table 1 and Figs. 2A and 3). This study has obtained dates consistent with, but much more refined than, previous $^{14}C$ work for the LMIA to II phases and for the Santorini VDL (*7–9*, *13*, *26–28*) and has demonstrated intra- and interlaboratory comparability. The date for the major Minoan eruption of Santorini (the VDL) is placed in the later 17th century B.C. (Fig. 2B): within the 95.4% confidence range of 1660–1613 B.C. [with 1639–1616 B.C. the most likely subrange looking at the 1σ ranges of 1656–1651 B.C. ($P$ = 0.113) and 1639–1616 B.C. ($P$ = 0.569)] from the analyses using IntCal04 or 1661–1605 B.C. from IntCal98 (Table 1). This age, from short-lived samples stored at the time the town

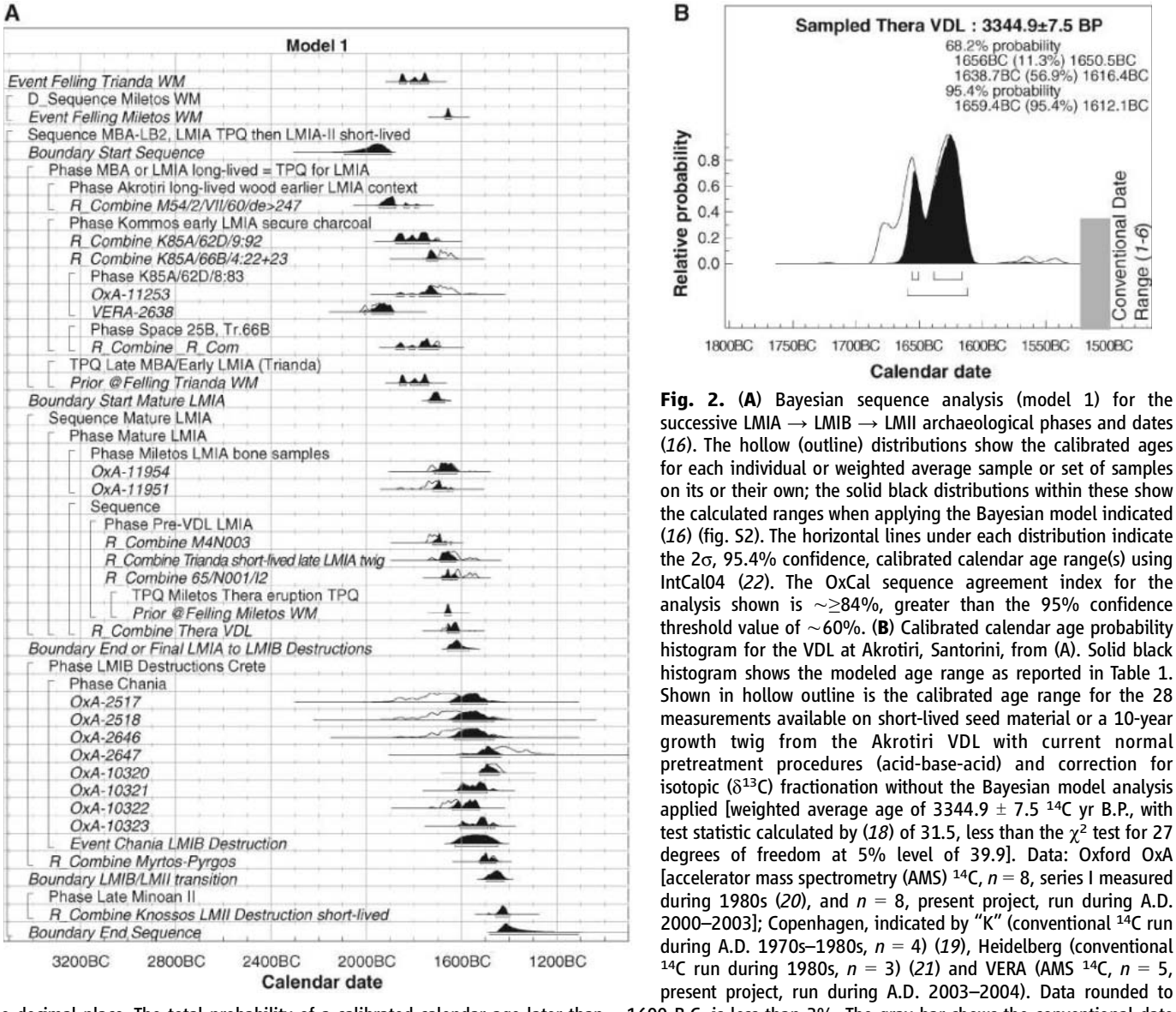


**Fig. 2.** (**A**) Bayesian sequence analysis (model 1) for the successive LMIA → LMIB → LMII archaeological phases and dates (*16*). The hollow (outline) distributions show the calibrated ages for each individual or weighted average sample or set of samples on its or their own; the solid black distributions within these show the calculated ranges when applying the Bayesian model indicated (*16*) (fig. S2). The horizontal lines under each distribution indicate the 2σ, 95.4% confidence, calibrated calendar age range(s) using IntCal04 (*22*). The OxCal sequence agreement index for the analysis shown is ~≥84%, greater than the 95% confidence threshold value of ~60%. (**B**) Calibrated calendar age probability histogram for the VDL at Akrotiri, Santorini, from (A). Solid black histogram shows the modeled age range as reported in Table 1. Shown in hollow outline is the calibrated age range for the 28 measurements available on short-lived seed material or a 10-year growth twig from the Akrotiri VDL with current normal pretreatment procedures (acid-base-acid) and correction for isotopic ($\delta^{13}C$) fractionation without the Bayesian model analysis applied [weighted average age of 3344.9 ± 7.5 $^{14}C$ yr B.P., with test statistic calculated by (*18*) of 31.5, less than the $\chi^2$ test for 27 degrees of freedom at 5% level of 39.9]. Data: Oxford OxA [accelerator mass spectrometry (AMS) $^{14}C$, $n$ = 8, series I measured during 1980s (*20*), and $n$ = 8, present project, run during A.D. 2000–2003]; Copenhagen, indicated by "K" (conventional $^{14}C$ run during A.D. 1970s–1980s, $n$ = 4) (*19*), Heidelberg (conventional $^{14}C$ run during 1980s, $n$ = 3) (*21*) and VERA (AMS $^{14}C$, $n$ = 5, present project, run during A.D. 2003–2004). Data rounded to one decimal place. The total probability of a calibrated calendar age later than ~1600 B.C. is less than 3%. The gray bar shows the conventional date range for the VDL of 1525–1500 B.C. (*1–6*).

at Akrotiri was abandoned just before the eruption, should either date the eruption within a year or two or set a close tpq for the eruption a few years later. It is consonant with the independent date of 1627–1600 B.C. at 2σ confidence (1621–1605 B.C. at 1σ) from $^{14}$C WM dating of an olive tree killed by the eruption (*29*) (Table 1 and fig. S8). Moreover, our data on short-lived samples from immediately before the eruption yield a contemporary to slightly older age range, demonstrating that this olive tree was last alive around or shortly after the final harvest year represented at Akrotiri, and thus its last preserved ring (bark) provides the best current date for the eruption.

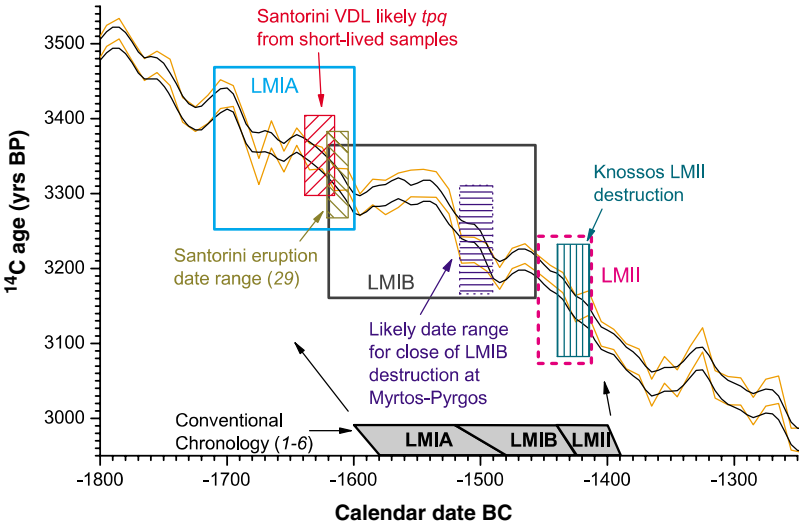


**Fig. 3.** Schematic representation of the $^{14}$C-derived Aegean early Late Bronze Age archaeological chronology summarized in Table 1, Fig. 2, and (*29*) shown against the Northern Hemisphere $^{14}$C calibration curve [IntCal04 from (*22*) shown in black as a ±1σ range, and IntCal98 from (*23*) shown in orange as a ±1σ range]. The chronology shows the 1σ ranges, or most likely subelement thereof, from Table 1 for events and a midpoint approximation for the transitions between phases. The latter is only a very approximate guide. Some complications are not addressed (and are not represented in our $^{14}$C evidence): for example the suggestion of a short post-Santorini-eruption final phase of LMIA (*1*) (which might move the start of LMIB lower, to around 1600 B.C.), and the transition date between LMIB and LMII is flexible and not well defined. The previous conventional Aegean chronology derived from the standard interpretation of the archaeological and art-historical associations (*1–6*) is shown below. The new $^{14}$C-derived chronology both begins the Late Bronze Age ~100 years earlier than previously accepted and also substantially lengthens the LMIA and (especially) LMIB and LMII cultural phases.

The conventional dates for the LMIA to II phases and for the Santorini eruption ~1525 to 1500 B.C., based on Egyptian contexts and associations, are inconsistent with our findings. This suggests either a defect in the conventional linkages to the Egyptian historical chronology in the mid–second millennium B.C. or a failing in the Egyptian chronology itself. Because the Egyptian historical chronology is widely considered relatively robust and in the 14th century B.C. (Amarna period) it correlates well both with the independent Mesopotamian historical chronology and $^{14}$C evidence (*30–32*), the problem more likely relates to the interculture linkages in the mid–second millennium B.C. [or, less likely, some chronological flaw affecting specifically mid–second millennium B.C. Egyptian dates (*16*)].

These findings imply that some previously hypothesized dates and associations for the Santorini eruption are now not likely. Suggested dates from tree-ring growth anomalies (*6*, *33–36*) remain as yet hypotheses lacking causal connection. The growth anomaly in a group of Aegean trees dated 1650 +4/–7 B.C. (*36*) now seems a little too early to be associated with Santorini. The question of the possibility of a relationship with the 1628 to 1627 B.C. tree-ring growth anomaly widely attested in the Northern Hemisphere (*33–35*) remains open but is challenged by the narrow date in (*29*). There is no positive evidence in favor of the suggestion of a 1525 or 1524 B.C. date (*6*), and it is incompatible with our findings and those of (*29*). Suggested dates ~1645 B.C. or otherwise from ice-core evidence (*8*, *37–39*) are unlikely or unclear

**Table 1.** Typical Bayesian analysis outcomes for model 1 (average 10 runs). The 2σ, 95.4%, confidence calibrated calendar date ranges B.C. calculated by the analysis shown in Fig. 2 are listed for a number of the key transitions or events or phases within the LMIA to LMII archaeological sequence. The 1σ (68.2% confidence) ranges with IntCal04 (*22*) are also shown in the first row of data marked with asterisks. Data rounded to the nearest whole year. Typical data given (each computer run of the model varies very slightly, with variation usually ≤2 years; quoted probabilities also vary slightly by run). Results against the IntCal98 $^{14}$C calibration data set, which was derived from similar underlying data but by a different modeling procedure (*23*), are also shown; the outcomes are very similar, which demonstrates the robustness of the conclusions irrespective of such minor changes in calibration data set. We further show results for (i) model 1 without any data from Santorini included, and the VDL calculated as an event within the sequence (fig. S5). The modeled placement for the VDL is entirely complementary with the data from Santorini, demonstrating that no offset effect applies to the Santorini data, which may therefore be used with confidence. (ii) Model 1 adding the Santorini olive tree WM information (*29*) (eruption event 1627–1600 B.C. at 2σ). The bottom row shows the conventional archaeologically derived dates (*1–6*) for comparison.

| | Transition to mature LMIA | Felling date Miletos oak | Akrotiri VDL | Transition end LMIA to LMIB | Myrtos-Pyrgos close of LMIB destruction | Knossos LMII destruction |
|---|---|---|---|---|---|---|
| Model 1, IntCal04 (*22*) | 1737–1673<br>1722–1695* | 1671–1644<br>1664–1652* | 1660–1612<br>1656–1651 (11.3%)*<br>1639-1616 (56.9%)* | 1659–1572<br>1647–1644 (3.1%)*<br>1642–1603 (65.1%)* | 1522–1456<br>1517–1491 (58.1%)*<br>1475–1467 (10.1%)* | 1457–1399<br>1439–1414* |
| Model 1, IntCal98 (*23*) | 1733–1665 | 1669–1646 | 1661–1605 | 1660–1567 | 1522–1487 (65.3%)<br>1482–1451 (30.1%) | 1489–1480 (3.6%)<br>1452–1394 (91.8%) |
| Model 1, no Santorini data, IntCal04 (*22*) | 1728–1643 | 1672–1645 | 1668–1585 | 1661–1553 | 1522–1456 | 1487–1481 (1.3%)<br>1457–1400 (94.1%) |
| Model 1, adding (*29*) data (fig. S8), IntCal04 (*22*) | 1737–1673 | 1671–1644 | 1654–1649 (3%)<br>1645–1611 (92.4%)<br>1σ: 1633–1617* | 1626–1562 | 1522–1457 | 1487–1480 (1.5%)<br>1458–1400 (93.9%) |
| Conventional chronology (*1–6*) | 1600/1580 | | 1525/1500 | 1520/1500/1480 | 1440/1430/1425 | 1400/1390 |

given recent critical discussions of associations, provenience of volcanic glass shards recovered, and the exact dates of the relevant ice-core layers (*39*–*41*). This age is also a little old given our findings and especially those in (*29*). Other work has argued that this 1645 B.C. record may instead reflect an eruption of Aniakchak (*40*). Allied with previous arguments for Santorini's relatively modest $SO_2$ output (*42*), the search for Santorini tephra in Arctic ice-core records should now focus on some of the (smaller) volcanic signals particularly in the late 17th century B.C., such as that of ~1622 to 1618 B.C. (Dye 3 ice core or Greenland Ice Core Project ice cores) (*37*).

Our findings are consonant with the so-called "high" Aegean chronology, which suggests a reinterpretation of some of the cultural linkages (*10*, *13*). The chronology places the formation and high point of the New Palace period of Crete (Middle Minoan III to LMIA), the linked Shaft Grave period of the Greek mainland (late Middle Helladic and Late Helladic I), and the closely associated Middle Cypriot III–Late Cypriot IA phase on Cyprus all before ~1600 B.C. These phases are thus contemporary with the world of the later Middle Bronze Age of the Levant and into the Second Intermediate Period in Egypt [~1650 or 1640 to 1540 or 1530 B.C., when northern Egypt was controlled by a Canaanite dynasty with links to the Levant (*43*)], not the earlier New Kingdom (18th Dynasty, ~1540–1295 B.C.) period of Egypt as long thought (regarding especially LMIA, Late Helladic I, and Late Cypriot IA). This chronology, and the 100-year shift in associations, in turn implies a reevaluation of previous culture-history and art-history assumptions and frameworks. For example, the well-known wall paintings unearthed at Akrotiri, Thera (*44*) need to be assessed in terms of contemporary 17th century B.C., and not later 16th century B.C., work in the east Mediterranean and Levant.

The difference between the Aegean $^{14}C$-based dates and archaeological dates is not constant across the second millennium B.C. Instead, by the LMII phase (late 15th century B.C.) the $^{14}C$ and archaeological dates are close, and other work indicates good agreement between them for the 14th to 13th centuries B.C. (*32*, *45*, *46*). Our evidence is compatible with the well-established conventional linkages between Old Palace (Middle Minoan, MM, IB to II) Crete and 12th and 13th Dynasty Egypt in the 19th and 18th centuries B.C. (*1*, *2*, *7*). The period (MMIII through LMII), originally interpolated between well-based archaeological associations linked with the Middle Kingdom (19th–18th centuries B.C.) and Amarna (mid–14th century B.C.) periods, alone needs revision, and LMIA to II is here suggested to date within the bounds ~1710–1410 B.C. (from likely 1σ ranges in Table 1 and Fig. 3) instead of ~1600 or 1580 to 1400 or 390 B.C. (*1*–*6*). (MMIII is left to be interpolated as a relatively short phase lying in the mid– to late 18th century B.C.) The date range for the later LMIB destruction horizon at Myrtos-Pyrgos 1522–1456 B.C. (2σ) and the likely LMIB-LMII transition ~1450 B.C. (Figs. 2 and 3) are consistent with the long-held correlation of part of this ceramic phase (or its mainland coeval phase of Late Helladic IIA) with the early New Kingdom in Egypt (which begins ~1550 or 1540 B.C.) and into the reign of Tuthmosis III (1479–1425 B.C.) (*1*–*3*, *5*, *7*, *11*). However, our dates show that the overall LMIB and LHIIA phases began earlier, and so were much longer, than previously thought. The overall New Palace period of Crete (MMIII to LMIB), when the island dominated Aegean trade and culture, is thus found to be a very long era (>250 years) (Fig. 3).

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### Supporting Online Material

www.sciencemag.org/cgi/content/full/312/5773/565/DC1
Materials and Methods
Figs. S1 to S8
Tables S1 to S3


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# Population Size Does Not Influence Mitochondrial Genetic Diversity in Animals

**Eric Bazin, Sylvain Glémin, Nicolas Galtier***

Within-species genetic diversity is thought to reflect population size, history, ecology, and ability to adapt. Using a comprehensive collection of polymorphism data sets covering ~3000 animal species, we show that the widely used mitochondrial DNA (mtDNA) marker does not reflect species abundance or ecology: mtDNA diversity is not higher in invertebrates than in vertebrates, in marine than in terrestrial species, or in small than in large organisms. Nuclear loci, in contrast, fit these intuitive expectations. The unexpected mitochondrial diversity distribution is explained by recurrent adaptive evolution, challenging the neutral theory of molecular evolution and questioning the relevance of mtDNA in biodiversity and conservation studies.

Genetic diversity is a central concept of evolutionary biology that has been linked to organismal complexity (*1*), ecosystem recovery (*2*), and species ability to respond to environmental changes (*3*). A lack of diversity is typically considered as evidence for a small or declining, potentially endangered population (*4*, *5*). Population genetics theory tells us that, for a neutral locus, the expected polymorphism at mutation-drift equilibrium is proportional to the effective population size, the equivalent number of breeders in an ideal, panmictic population. Other factors can of course affect the genetic polymorphism, including population structure (*6*), population bottlenecks (*3*), and natural selection [either directly or through genetic linkage (*7*, *8*)], life cycle (*9*), and mating systems (*10*). These multiple influences complicate any attempt to interpret the genetic diversity of one particular species in terms of population size (*11*). Population size, however, presumably varies by several orders of magnitude between species and taxa, so that one would typically predict that abundant species should be, on average, more polymorphic than scarce ones despite the noise introduced by other evolutionary forces.

Meta-analyses of allozyme polymorphism studies were mostly consistent with this theoretical prediction (*12*, *13*). In particular, invertebrate animals were found to be more polymorphic, on average, than vertebrates (*13*). It was noted, however, that the expected proportional relationship between diversity and effective population size was rarely met (*14*). DNA-based markers have now replaced allozymes in population genetics studies. Among these, the supposedly nonrecombining and evolutionary nearly neutral mitochondrial DNA (mtDNA) has been the most widely used marker of population history and diversity (*15*, *16*), the general belief being that mtDNA diversity should reflect effective population size more accurately than allozymes (*17*). In this study, we approach the taxonomic and ecological determinants of effective population size by analyzing the distribution of the genetic polymorphism across animal taxa, focusing on mtDNA and comparing it to allozymes and nuclear DNA data.

Three exhaustive within-species polymorphism data sets were used: an allozyme data set (912 species) taken from the compilation by Nevo *et al*. (*12*), a nuclear sequence data set (417 species), and a mitochondrial sequence data set (1683 species), the latter two both built from the Polymorphix database (*18*, *19*). We first calculated the average genetic diversity in eight largely represented animal taxa (hereafter called "groups"). The allozyme and nuclear data sets yielded highly similar results (Fig. 1): The average within-species diversity in all four invertebrate groups was higher than that of vertebrates, mollusks being the most diverse and mammals the least diverse, on average. This is essentially in agreement with our intuition about species abundance in these taxa. The mtDNA data diversity, however, was highly variable between species within a group, but remarkably homogeneous between groups (Fig. 1). Insect or mollusk species did not appear more polymorphic, on average, than mammals or birds, contradicting our prior beliefs about relative population sizes in these taxa. The average invertebrate mtDNA diversity (7.67%) was not appreciably different from the vertebrate one (7.99%), whereas the nuclear invertebrate average (2.46%) was four times as high as the vertebrate one (0.60%).

A series of within-group analyses were conducted to examine the influence of specific ecological variables (Table 1). Allozyme data again agreed with our intuition about population sizes: Among mollusks, the terrestrial pulmonates were substantially less polymorphic than marine bivalves or gastropods, consistent with the enormous dispersal potential of the latter; among crustaceans, the microscopic, planktonic branchiopods (e.g., *Artemia* and *Daphnia*) appeared much more diverse than the larger decapods (shrimps, lobsters, and crabs); among fish, marine species showed a significantly higher heterozygosity than the geographically restricted freshwater species. The mtDNA diversity, in contrast, failed to reflect these differences in average population size. Again, a homogeneous average nucleotide diversity was found, irrespective of body size and ecology (Table 1). Freshwater fish species were even significantly more polymorphic than marine ones.

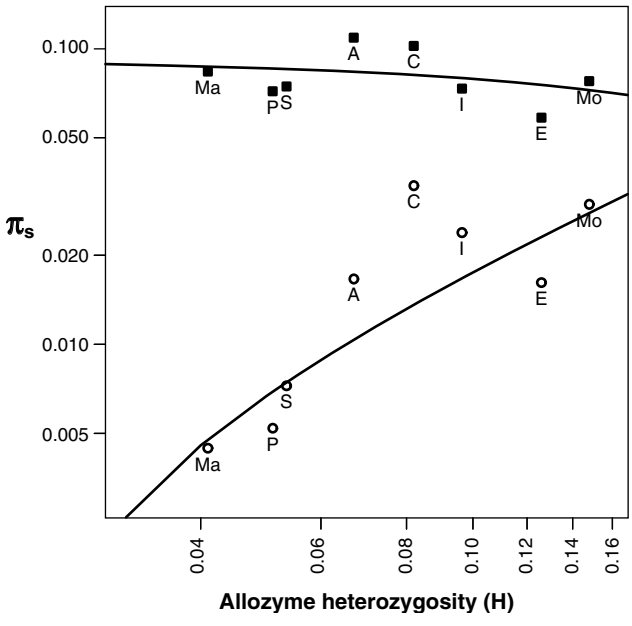


**Fig. 1.** Average allozymic, nuclear DNA, and mtDNA diversity in eight animal taxa. *x* axis: allozyme average heterozygosity. *y* axis: circles, nuclear DNA average synonymous diversity (kendall test: $\tau = 0.87$, $P < 0.05$); squares, mtDNA average synonymous diversity (kendall test: $\tau = -0.14$, not significant). Ma: Mammalia (allozymes: 184 species; nuclear: 30 species; mtDNA: 350 species); S: Sauropsida (reptiles and birds: 116, 20, 378); A: Amphibia (61, 4, 96); P: Pisces (bony fish and cartilaginous fish: 183, 22, 270); I: Insecta (156, 73, 511); C: Crustacea (122, 2, 78); E: Echinodermata (sea stars and urchins: 15, 14, 47); and Mo: Mollusca (46, 9, 125). The nuclear averages of the little-represented Amphibia (four species) and Crustacea (two species) are shown but were not used for the statistical test.

CNRS UMR 5171–Génome, Populations, Interactions, Adaptation–Université Montpellier 2 34095 Montpellier Cedex 5, France.

*To whom correspondence should be addressed. E-mail: galtier@univ-montp2.fr

Variations in mitochondrial mutation rate among phyla could be invoked to explain the discrepancy between animal mtDNA diversity and effective population size. The mutation rate, however, would have to be inversely related to population size throughout animal taxa to explain the data—a pattern very unlikely to appear by chance. Demographic stochasticity, e.g., recurrent population bottlenecks, could remove the effect of equilibrium population size on genetic diversity (*20*). Demographic effects, however, should affect the nuclear genome as well, which is not what we observe. Natural selection, either purifying or adaptive, must therefore be invoked to explain the locus-specific behavior of mtDNA.

Purifying selection against deleterious mutations (so-called background selection) decreases the diversity at linked loci through hitch-hiking. The strength of this effect depends on the distribution of fitness effects among mutations, and one generally still expects an increase of diversity with population size under background selection (*21*), which is not consistent with the homogeneous mtDNA diversity distribution. Our analytical results confirmed this statement: The conditions under which background selection can lead to a more or less independent relationship between diversity and effective population size appear implausible (fig. S2).

The mtDNA pattern, however, appears to be in good agreement with the hypothesis of recurrent fixation of advantageous mutations leading to frequent loss of variability at linked loci (*7*, *22*), a process recently named "genetic draft" by Gillespie (*23*). The population number of advantageous mutations per generation obviously increases with population size and compensates the decrease of genetic drift in Gillespie's (*24*) simulations, which predict an essentially flat, even negative, relationship between genetic diversity and population size. The gene-dense, nonrecombining context of the animal mitochondrial genome maximizes the potential impact of the genetic draft, as compared with that of the nuclear genome (*25*).

To firmly distinguish between the two selective models, we examined the pattern of nucleotide substitution between species. The neutrality index (NI) (*26*) was first calculated when outgroup sequences were available. This index aims at comparing the ratio of nonsynonymous (amino acid–changing) to synonymous (silent) changes within species ($\pi_N/\pi_S$) and between species ($d_N/d_S$): NI is 1 when evolution is neutral, greater than 1 under purifying selection, and less than 1 in the case of adaptation. A significant shift toward values less than 1 was detected in invertebrate mtDNA loci, consistent with the adaptive hypothesis (Fig. 2 and fig. S1).

This result, limited to the genes for which polymorphism data are available, was confirmed by a whole-genome mitochondrial analysis. The $d_N/d_S$ ratio was calculated for the 13 mitochondrial protein-coding genes in various animal taxa (Table 2). The average genomic $d_N/d_S$ was significantly higher in invertebrates than in vertebrates. This is not consistent with a model invoking solely purifying selection, because the rate of fixation of deleterious mutations is expected to decrease with population size. Observing a higher rate of nonsynonymous substitution, but not a higher level of diversity, in large populations strongly corroborates the hypothesis that positive selection drives mitochondrial evolution in animals: Neither negative selection (which should decrease $d_N/d_S$ and increase NI) nor a relaxation of constraints (which should increase the diversity) can explain this pattern. The additional amino acid substitutions detected in invertebrates would correspond to adaptive changes, plus the deleterious ones hitch-hiking to fixation—the rate of deleterious substitution is expected to increase with population size in the genetic draft model (*24*).

This study reveals that the mitochondrial diversity of a given animal species does not reflect its population size: No correlation between mtDNA polymorphism and species abundance could be detected, despite the large body of data analyzed. Nuclear data, in contrast, are fairly consistent with intuitive expectations. We conclude that natural selection acting on mtDNA contributes to homogenization of the average diversity among groups, in agreement with the genetic draft theory. mtDNA appears to be anything but a neutral marker (*16*) and probably undergoes frequent adaptive evolution, e.g., direct

**Table 1.** Ecological determinism of allozyme and mtDNA genetic diversity. The numbers of species used are shown in parentheses.

| Taxon | | Allozymes (H, %) | mtDNA ($\pi_s$, %) |
|---|---|---|---|
| Fish | Freshwater | 4.7 (71) | 8.7** (123) |
| | Marine | 6.1* (65) | 3.7 (51) |
| Crustaceans | Large benthic | 4.6 (81) | 10.1 (26) |
| | Small planktonic | 21.0* (8) | 5.8 (6) |
| Mollusks | Terrestrial | 7.4 (23) | 7.8 (8) |
| | Marine | 30.0** (17) | 5.6 (34) |

*$P < 0.05$ (Student's $t$ test). **$P < 0.01$ (Student's $t$ test).

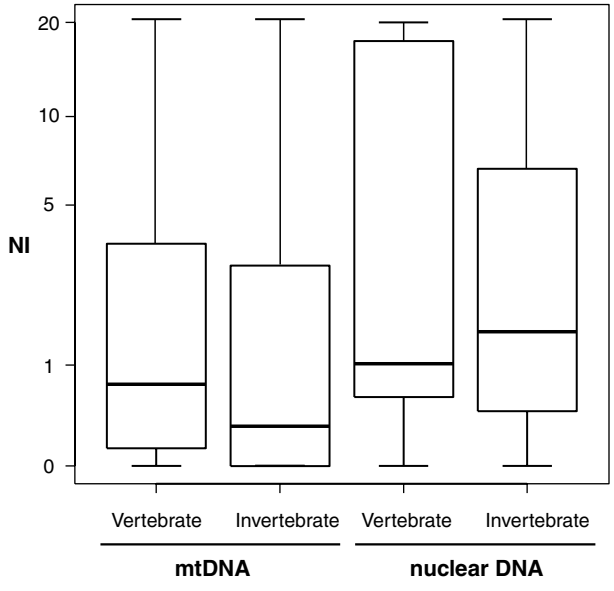


**Fig. 2.** Neutrality index (NI) distributions (logarithmic scale). Medians are indicated by thick horizontal bars. Boxes include 50% of the distributions. The invertebrate mtDNA median NI (0.42) is significantly lower than the vertebrate one (0.88; $P < 10^{-3}$, Mann-Whitney test). NI values greater than 20 were forced to 20 for clarity. Low-frequency (<0.125) polymorphic sites were excluded from the analysis.

**Table 2.** Mitochondrial genomic $d_N/d_S$ ratio in animals.

| Taxon | Data sets | $d_N/d_S$ |
|---|---|---|
| | *Vertebrates* | |
| Mammalia | 21 | 0.080 |
| Sauropsida | 9 | 0.121 |
| Amphibia | 12 | 0.086 |
| Teleostei | 44 | 0.065 |
| Chondrichthyes | 2 | 0.077 |
| Average | | 0.086 |
| | *Invertebrates* | |
| Insecta | 4 | 0.198 |
| Crustacea | 5 | 0.084 |
| Mollusca | 2 | 0.122 |
| Echinodermata | 1 | 0.106 |
| Nematoda | 2 | 0.219 |
| Chelicerata | 6 | 0.138 |
| Platyhelminthes | 1 | 0.140 |
| Urochordata | 1 | 0.188 |
| Cnidaria | 2 | 0.167 |
| Average | | 0.151 ** |

**$P < 0.01$ (Student's $t$ test).

selection on the respiratory machinery (*27*), nucleo-cytoplasmic coadaptation (*28*), two-level selection (*29*), or adaptive introgression, perhaps hitchhiking with a maternally transmitted parasite (*30*). mtDNA diversity is essentially unpredictable and will, in many instances, reflect the time since the last event of selective sweep, rather than population history and demography. Low-diversity mitochondrial lineages, typically disregarded as important from a conservation standpoint, might sometimes correspond to recently selected, well-adapted haplotypes to be preserved.

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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/312/5773/570/DC1
Materials and Methods
Figs. S1 to S5
References and Notes


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# Proapoptotic BAX and BAK Modulate the Unfolded Protein Response by a Direct Interaction with IRE1α


**Claudio Hetz,[1,2*] Paula Bernasconi,[1] Jill Fisher,[1] Ann-Hwee Lee,[2] Michael C. Bassik,[1] Bruno Antonsson,[4] Gabriel S. Brandt,[5] Neal N. Iwakoshi,[2] Anna Schinzel,[1] Laurie H. Glimcher,[2,3*] Stanley J. Korsmeyer[1]†**



**Accumulation of misfolded protein in the endoplasmic reticulum (ER) triggers an adaptive stress response—termed the unfolded protein response (UPR)—mediated by the ER transmembrane protein kinase and endoribonuclease inositol-requiring enzyme–1α (IRE1α). We investigated UPR signaling events in mice in the absence of the proapoptotic BCL-2 family members BAX and BAK [double knockout (DKO)]. DKO mice responded abnormally to tunicamycin-induced ER stress in the liver, with extensive tissue damage and decreased expression of the IRE1 substrate X-box–binding protein 1 and its target genes. ER-stressed DKO cells showed deficient IRE1α signaling. BAX and BAK formed a protein complex with the cytosolic domain of IRE1α that was essential for IRE1α activation. Thus, BAX and BAK function at the ER membrane to activate IRE1α signaling and to provide a physical link between members of the core apoptotic pathway and the UPR.**


Cell viability depends on the functional and structural integrity of intracellular organelles. Multidomain proapoptotic BAX and BAK proteins function in concert as essential gateways to intrinsic cell death pathways operating at mitochondria (*1*). Several anti- and proapoptotic BCL-2 family members also localize to the ER and modulate steady-state calcium homeostasis (*2–4*). In higher eukaryotes, ER stress stimulates three distinct UPR signaling pathways through sensors that include IRE1α (also described as inositol-requiring transmembrane kinase and endonuclease 1α), PERK (protein kinase–like ER kinase), and ATF6 (activation of transcription factor 6) (*5*, *6*). IRE1α is a serine-threonine protein kinase and endoribonuclease that, on activation, initiates the unconventional splicing of the mRNA encoding X-box–binding protein 1 (XBP-1) (*7–9*). Spliced XBP-1 is a potent transcriptional activator that increases expression of a subset of UPR-related genes (*10*). The cytosolic domain of activated IRE1α binds the adaptor protein TRAF2 [tumor necrosis factor (TNF)–associated factor 2], and triggers the activation of the c-Jun N-terminal kinase (JNK) signaling pathway (*11*, *12*). Activated PERK directly phosphorylates and inhibits the translation initiation factor eIF2α (thus decreasing protein loading into the ER) and induces expression of the transcription factor ATF4, which increases expression of certain UPR genes such as *Chop* or *GADD153* and *Grp78* or *BiP* (*5*, *7*). BiP is a chaperone that maintains PERK and IRE1α in an inactive state. However, in cells undergoing ER stress, BiP preferentially binds to misfolded proteins, thereby releasing the stress sensors to undergo activation by homodimerization and autophosphorylation (*13*, *14*).

Double knockout (DKO) cells from BAX-BAK–deficient mice are resistant to proapoptotic agents that induce the UPR (*1*) and also show a defect in steady-state ER calcium homeostasis under nonapoptotic conditions (*3*). A validated in vivo model for ER stress uses intraperitoneal injection of tunicamycin (Tm, an inhibitor of N-linked glycosylation) (*15–17*). This treatment triggers a stress response in the liver and kidney that causes extensive cell death in these organs after several days of treatment. Most DKO mice (more than 90%) die during embryogenesis (*18*), as a result of developmental defects. We generated a conditional BAX-BAK DKO model in which a *bax* allele flanked with LoxP sites was targeted in *bak*-null embryonic stem cells (*19*). To achieve inducible deletion of *Bax* in adulthood, MxCre$^{+}$*bax*$^{fl/}$*bak*$^{-/-}$ and control MxCre$^{+}$*bax*$^{+/-}$*bak*$^{-/-}$ mice were treated with poly(IC). Mice were subsequently injected with Tm (1 μg/g body weight) and


[1]Howard Hughes Medical Institute, Dana–Farber Cancer Institute, and Harvard Medical School, Boston, MA 02115, USA. [2]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA 02115, USA. [3]Department of Medicine, Harvard Medical School, Boston, MA 02115, USA. [4]Serono Pharmaceutical Research Institute, 14 Chemin des Aulx, 1228 Plan les Ouates, Switzerland. [5]Departments of Biochemistry and Chemistry, Rosenstiel Center, Brandeis University, Waltham, MA 02454, USA.

†Deceased.

*To whom correspondence should be addressed. E-mail: lglimche@hsph.harvard.edu (L.H.G.); chetz@hsph.harvard.edu. (C.H.)

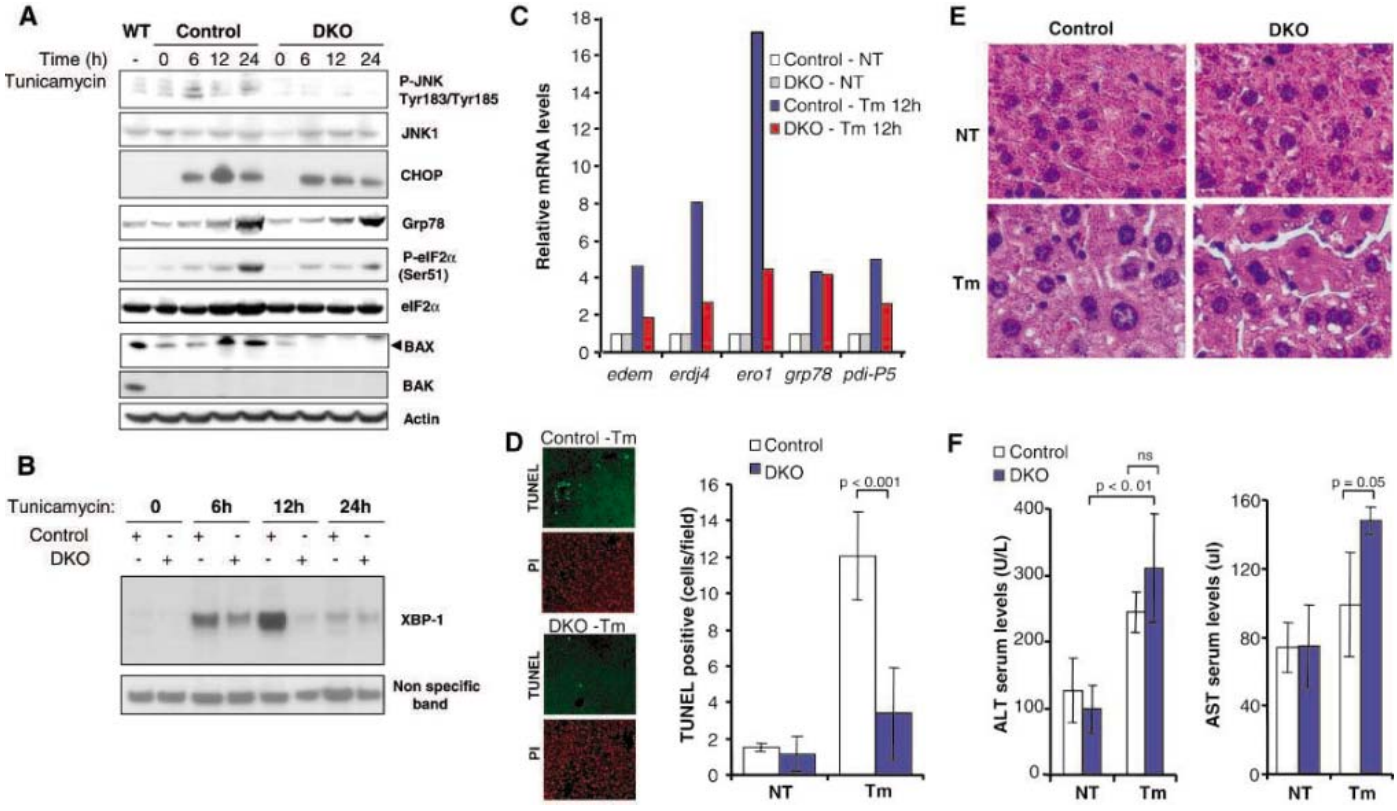


**Fig. 1.** Diminished XBP-1 induction and JNK phosphorylation in DKO livers of mice injected with Tm. MxCre$^{+}$*bax*$^{+/-}$*bak*$^{-/-}$ (control) and MxCre$^{+}$*bax*$^{fl/-}$*bak*$^{-/-}$ (DKO) mice were treated with poly(IC) to induce *bax* deletion. Animals were injected with Tm and killed after 6, 12, or 24 hours. (**A**) Expression levels of phospho-JNK, JNK1, CHOP/GADD153, BiP (Grp78), BAX, BAK, phospho-eIF2α, eIF2α, and actin were analyzed by Western blot in total liver extracts. WT livers from untreated mice were used as a positive control for BAK expression. (**B**) XBP-1 expression levels were analyzed in nuclear extracts of liver samples obtained in (A). A nonspecific band from the same Western blot is shown as a loading control. (**C**) Relative mRNA levels of *edem*, *erdj4*, *ero1*, *pdi-P5*, and *bip* were quantified by real-time polymerase chain reaction (PCR) in total cDNA obtained from the liver of control and DKO mice injected with Tm for 12 hours. (**D**) TUNEL staining of liver sections from control and DKO mice 4 days after Tm injection. Representative images of tissue from Tm-treated animals are presented. Green fluorescence, TUNEL staining; red fluorescence, propidium iodide (PI) staining. Graphically displayed results represent means and SD from the quantification of five different animals per group. (**E**) Hematoxylin-and-eosin staining of samples analyzed in (D). Of note, increased cytosolic vacuolation was observed in MxCre$^{+}$*bax*$^{fl/-}$*bak*$^{-/-}$ mice compared with control animals ($n$ = at least five animals per group). (**F**) In parallel, serum alanine aminotransaminase (ALT) and aspartate aminotransferase (AST) levels were determined. Results represent means and SDs from three to four different animals before and after Tm injections. Where indicated, $P$ values for statistical analysis were obtained using Student's $t$ test (ns, not significantly different).

killed 6, 12, or 24 hours after injection. Similar expression levels of phospho-eIF2α, CHOP, and BiP were observed in control or MxCre$^{+}$*bax*$^{fl/-}$*bak*$^{-/-}$ livers after Tm injection (Fig. 1A), indicating unperturbed PERK signaling. However, JNK phosphorylation was decreased in MxCre$^{+}$*bax*$^{fl/-}$*bak*$^{-/-}$ livers (Fig. 1A). Six hours after Tm injection, increased abundance of XBP-1 was observed by Western blot analysis of lysates prepared from nuclear fractions of control liver extracts, but only a small increase in the amount of XBP-1 was detected in extracts from MxCre$^{+}$*bax*$^{fl/-}$*bak*$^{-/-}$ liver (Fig. 1B), and almost no XBP-1 was evident 12 hours after injection (Fig. 1B). Expression of XBP-1 target genes (*10*) *edem*, *erdj4*, and *ero1L*, as well as protein disulfide isomerase *pdi-P5*, but not *bip* (a negative control), was reduced in MxCre$^{+}$*bax*$^{fl/-}$*bak*$^{-/-}$ livers compared with control livers (Fig. 1C). The number of cells that showed terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL-positive cells) was significantly decreased in MxCre$^{+}$*bax*$^{fl/-}$*bak*$^{-/-}$ liver compared with those of control animals (Fig. 1D), consistent with the lack of mitochondrial expression of BAX and BAK. Despite decreased apoptosis, histological analysis of the liver (Fig. 1E) and elevated alanine aminotransaminase and aspartate aminotransferase activities in serum (Fig. 1F) revealed increased tissue damage in MxCre$^{+}$*bax*$^{fl/-}$*bak*$^{-/-}$ animals after Tm injection, consistent with impaired XBP-1 expression (*20*). Thus, the phenotype observed in BAX-BAK–deficient mice after Tm injection is indicative of cellular dysfunction rather than cell death. A normal stress response was observed in the kidney, where low deletion efficiency of the floxed allele provides an internal control (fig. S1). Overall, these data suggest that BAX and BAK have a crucial role in adaptation responses to ER stress in vivo through modulating IRE1α to XBP-1 signaling.

To explore further the signaling pathways by which BAX and BAK modulate the UPR, we initiated an ER stress response with Tm in wild-type (WT) and DKO murine embryonic fibroblasts (MEFs) (*1*) and analyzed the time course of signaling events initiated by IRE1α and PERK. XBP-1 expression was decreased in DKO cells undergoing ER stress compared with WT MEFs. Little or no phosphorylation of JNK at Tyr$^{183}$ and Tyr$^{185}$ and of its upstream kinase ASK1 (apoptosis signal–regulating kinase) was observed after Tm treatment of DKO cells (Fig. 2A). In contrast, tumor necrosis factor–α (TNF-α) (*21*) caused normal JNK phosphorylation in DKO cells (fig. S2A). In summary, DKO cells displayed a phenotype similar to that of IRE1α-deficient cells (fig. S2B) (*7*, *10*).

BAX and BAK deficiency did not alter PERK activation, as observed from comparable

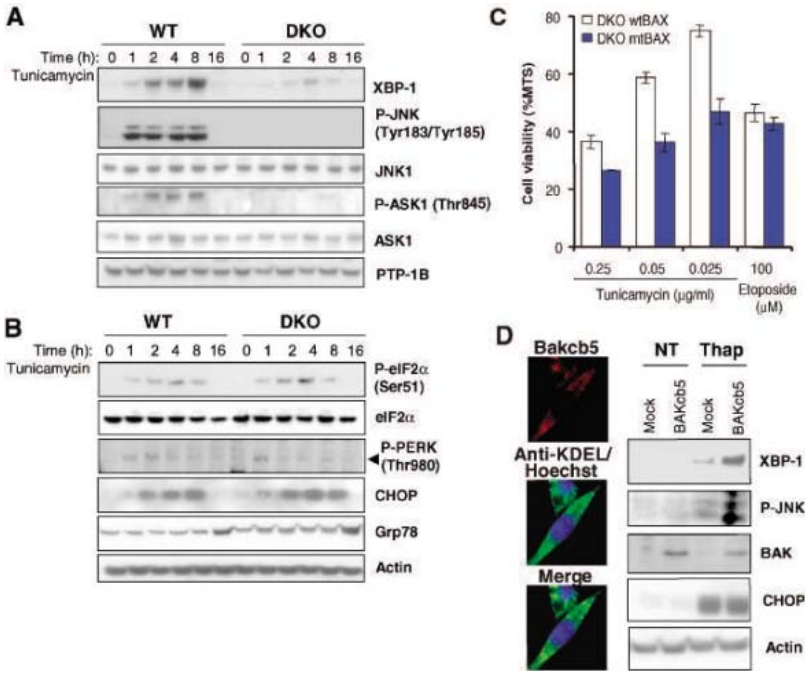


**Fig. 2.** BAX and BAK regulate IRE1α signaling in cells undergoing ER stress. (**A**) WT and DKO MEFs were treated with Tm (2.2 μg/ml) for indicated time points, and expression levels of XBP-1, phospho-JNK, phospho-ASK1, and PTP-1B were determined by Western blot. As a loading control, total JNK and ASK1 levels were assessed. (**B**) PERK signaling events were studied in cells treated as above with Tm. Expression levels of phospho-eIF2α, total eIF2α, phospho-PERK, CHOP, BiP, and actin were determined by Western blot. (**C**) DKO cells stably expressing WT BAX (wtBAX) or mtBAX were treated with the indicated concentration of Tm or etoposide, and cell viability was determined after 24 hours of incubation by the soluble tetrazolium salt, MTS, cytotoxicity assay. Differences in Tm treatment between cell types were statistically significant ($P < 0.001$), as analyzed by two-way analysis of variance (ANOVA). (**D**) BAKcb5 was transiently expressed in DKO cells for 48 hours in the presence of zVAD-fmk (10 μM) and then treated with thapsigargin (Thap) for 4 hours. Expression levels of XBP-1, phospho-JNK, BAKcb5, CHOP, and actin were determined by Western blot. (Left) Controls—the subcellular localization of BAKcb5 was assessed by immunofluorescence (red), costaining the ER with a KDEL antibody (green) and the nucleus with Hoechst (blue). All results are representative of at least three independent experiments.

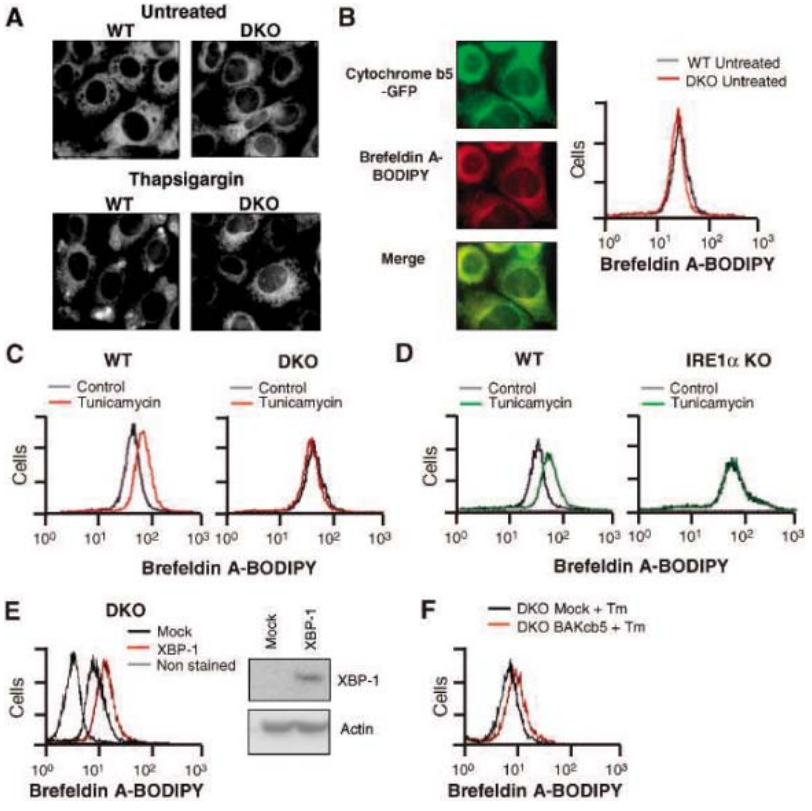


**Fig. 3.** Impaired expansion of the secretory pathway in DKO cells undergoing ER stress. (**A**) WT and DKO cells stably expressing cytochrome b5–GFP were treated with thapsigargin (1.5 μM) for 2.5 hours or left untreated, and the morphology of the ER (GFP fluorescence) was assessed. (**B**) DKO cells expressing cytochrome b5–GFP (green) were stained with brefeldin A–BODIPY (red), and fluorescence emission was analyzed (left panel). WT or DKO cells were stained with brefeldin A–BODIPY, and basal fluorescence emission was analyzed by FACS (right). (**C**) WT or DKO cells were treated with Tm (2.2 μg/ml) for 2.5 hours and then analyzed by FACS after staining with brefeldin A–BODIPY. (**D**) For comparison, IRE1α knockout (KO) cells or WT control cells were analyzed as described in (C). (**E**) DKO cells were transduced with a retroviral expression vector for spliced XBP-1 or empty vector (mock) and, after 48 hours of selection with hygromycin, cells were stained with brefeldin A–BODIPY and analyzed by FACS. Autofluorescence of unstained cells is shown. (Right) Expression levels of XBP-1 and actin were determined by Western blot in the same samples. (**F**) DKO cells transiently expressing BAKcb5 for 48 hours and then treated with Tm (2.2 μg/ml) for 2.5 hours were analyzed by FACS after brefeldin A–BODIPY staining. Experiments were performed in the presence of zVAD-fmk (10 μM). All results are representative of at least three independent experiments performed in duplicates.

phosphorylation of PERK ($Thr^{980}$) and eIF2α ($Ser^{51}$) in WT and DKO cells (Fig. 2B). In agreement with this result, the expression of PERK downstream targets, such as CHOP and BiP, were similar in WT and DKO cells (Fig. 2B) as previously described (*22*, *23*). As an additional control, UPR activation was assessed in cells lacking the antiapoptotic protein BCL-2. No defect in XBP-1 and CHOP induction was observed in Tm-treated BCL-2–deficient cells (fig. S2C). Thus, the defects in IRE1α activity observed in DKO cells are likely not a conse-

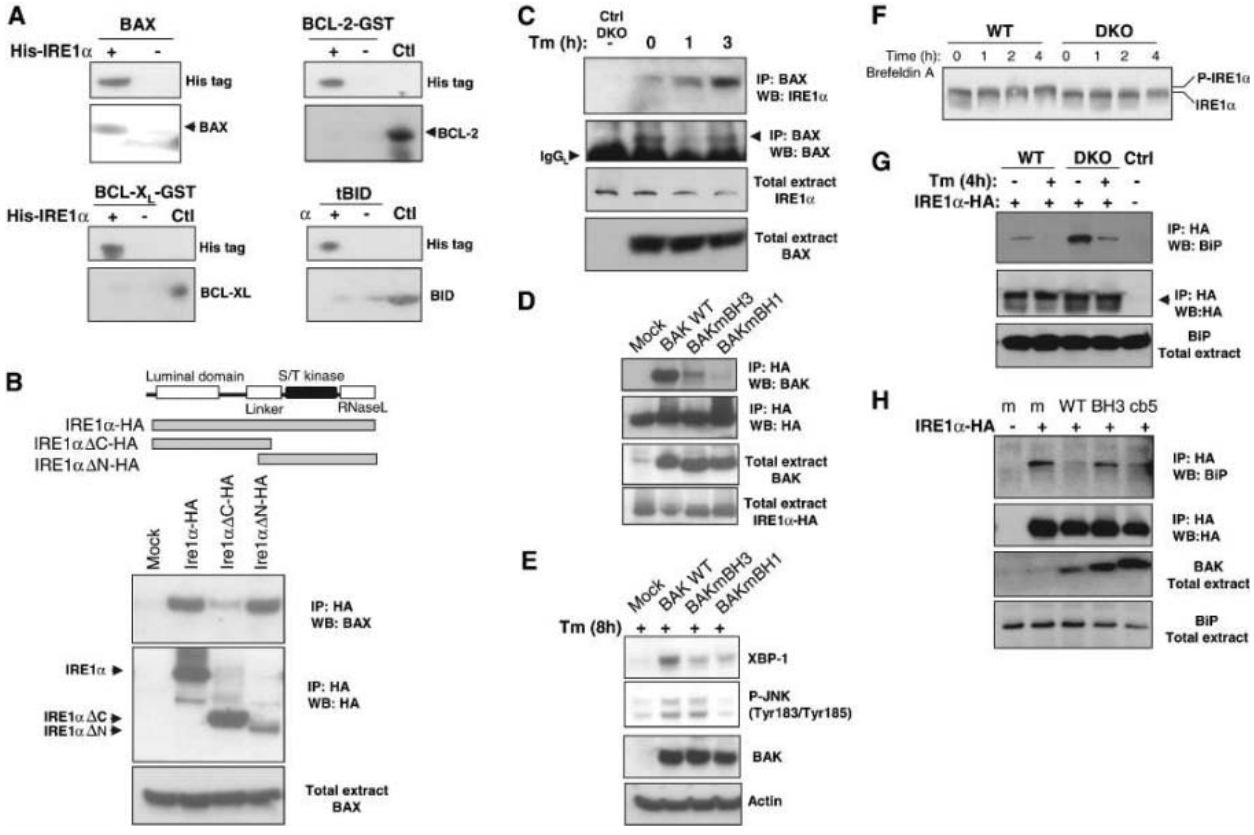


**Fig. 4.** Protein complex formation between BAX/BAK and IRE1α. (**A**) The binding of recombinant BCL-2–related proteins with a His-tagged version of the cytosolic domain of IRE1α (His-IRE1α) was investigated by pull-down assay with Ni-agarose and analyzed by Western blot. Control experiments were performed in the absence of IRE1α. As positive controls, total BCL-2 recombinant proteins (0.5 μg) were loaded where indicated. (**B**) Cell extracts from WT MEFs stably expressing full-length IRE1α-HA, the N-terminal region (IRE1αΔC-HA), the C-terminal region (IRE1αΔN-HA), or empty vector (mock) were immunoprecipitated with agarose containing antibody against hemagglutinin (HA), and association with endogenous BAX was assessed by Western blot. (**C**) WT MEFs were treated with Tm (2.2 μg/ml) for the indicated times or left untreated, and BAX was immunoprecipitated. Association with endogenous IRE1α was assessed by Western blot. Control immunoprecipitation experiments were performed with DKO cell extracts (Ctrl DKO). (**D**) DKO cells stably expressing IRE1α-HA were transiently infected with retroviruses encoding BAK WT, BAKmBH3, BAKmBH1, or empty vector (mock) for 48 hours in the presence of zVAD-fmk (10 μM), and their coimmunoprecipitation with IRE1α-HA was assessed with HA antibody coupled to agarose. (**E**) In parallel, DKO cells reconstituted with vectors as described in (D) were treated with Tm for 8 hours, and expression levels of XBP-1, phospho-JNK, BAK, and actin were determined by Western blot. (**F**) WT and DKO cells stably expressing HA-IRE1α were treated with brefeldin A (20 μM) for the indicated times, and the electrophoretic pattern of IRE1α was analyzed by Western blot (phosphorylated IRE1α, P-IRE1α). (**G**) WT and DKO cells stably expressing IRE1α-HA or empty vector (Ctrl) were treated with Tm for 4 hours or left untreated, and IRE1α was immunoprecipitated for analysis of BiP binding. (**H**) DKO cells stably expressing IRE1α-HA were transiently infected with retroviruses encoding BAK WT (WT), BAKmBH3 (BH3), BAKcb5 (cb5), or empty vector (m) for 48 hours in the presence of zVAD-fmk (10 μM), and the coimmunoprecipitation of BiP with IRE1α-HA was assessed. Control immunoprecipitation experiments were performed in WT cells not expressing IRE1α-HA.

quence of imbalanced expression of anti- and proapoptotic proteins.

BAX and BAK regulate apoptosis initiated at the mitochondria. To assign the site of BAX and BAK action in modulating IRE1α signaling, we performed organelle-specific reconstitution assays. Expression of a mitochondria-targeted form of BAX (mtBAX) (*3*) did not restore activation of the IRE1α pathway as measured by JNK activation or XBP-1 expression (fig. S3A), although it did restore the susceptibility of DKO cells to ER stress–mediated apoptosis (*3*) (fig. S3B). DKO cells reconstituted with WT BAX or mtBAX sensed apoptotic signals at the mitochondria as reflected by similar rates of apoptosis induced by the DNA damage agent etoposide (Fig. 2D) or by overexpression of tBID (truncated, active fragment of BID) (fig. S3C). Cells expressing mtBAX were more sensitive to ER stress than were cells expressing WT BAX, which suggests that BAX expression at the ER membrane may have a positive effect on adaptation to ER stress (Fig. 2C and fig. S3D). We reconstituted DKO cells with BAKcb5, a BAK mutant in which the transmembrane domain is replaced by the ER targeting sequence of cytochrome b5 (*23*), in the presence of caspase inhibitors to minimize toxic effects. BAKcb5 expression increased amounts of XBP-1 and phospho-JNK in DKO cells undergoing ER stress and did not affect CHOP expression (Fig. 2D). These findings indicate that BAK expression at the ER membrane is required for IRE1α signaling. Altered calcium homeostasis appeared not to be responsible for the defects in IRE1α signaling, because restoration of ER calcium content by SERCA (sarcoplasmic or endoplasmic reticulum calcium adenosine triphosphatase) overexpression (*3*) did not restore stress-induced expression of XBP-1 or JNK activation in DKO cells (fig. S3A), although these cells recovered susceptibility to calcium-mediated apoptosis (*3*).

In plasma cells, XBP-1 induced the expression of multiple secretory pathway genes, increased cell size, initiated biogenesis of the ER, and elevated total protein synthesis (*24*–*26*). We assessed ER morphology in DKO cells expressing a cytochrome b5–green fluorescent protein (GFP) fusion protein that allows visualization of the ER (*27*) (fig. S4A). BAX and BAK deficiency prevented the appearance of morphological changes in the ER such as

vacuolization and redistribution of the ER triggered in response to thapsigargin treatment (Fig. 3A). This effect was independent of caspase activation or calcium release (fig. S4B). Secretory pathway expansion was quantified by fluorescence-activated cell sorting (FACS) in living cells stained with a red fluorescent version of brefeldin A (*24*) that labels the ER and Golgi compartments (Fig. 3B, left). ER-Golgi content was similar in WT and DKO cells under unstressed conditions (Fig. 3B, right). However, increased brefeldin A–BODIPY (boron dipyrromethene difluoride) staining was observed in WT but not DKO cells undergoing ER stress (Fig. 3C and fig. S3C). The phenotype of IRE1α knockout cells resembled that observed in DKO cells (Fig. 3D and fig. S3D). DKO cells retained the ability to expand the ER-Golgi network, because transient expression of the spliced form of XBP-1 in DKO cells triggered this process (Fig. 3E), further implicating BAX and BAK as upstream regulators of the IRE1α and XBP-1 pathway. Moreover, expression of BAKcb5, but not mtBAX or SERCA (fig. S3E), restored the ability of DKO cells to expand the secretory pathway under ER stress conditions (Fig. 3F).

The expression of proteins that regulate IRE1α activation, such as PTP-1B (Fig. 2A) and BiP (Fig. 2B), was not altered in DKO cells, which raises the possibility that BCL-2–related proteins might directly interact with IRE1α. We therefore tested a His-tagged version of the cytosolic region of human IRE1α (His-cytIre1α, amino acids 468 to 977) for its ability to bind recombinant BAX, tBID, a fusion protein of BCL-2 with glutathione *S*-transferase (GST), or BCL-$x_L$–GST. Isolation of complexes with nickel-agarose revealed an interaction between His-cytIRE1α and BAX (Fig. 4A and fig. S5A). No binding was observed between His-cytIRE1α and BCL-2, BCL-$x_L$, or proapoptotic tBID (Fig. 4A).

Coimmunoprecipitation experiments using lysates from cells transfected with IRE1α hemagglutinin-tagged cDNA (IRE1α-HA) showed an association of IRE1α with endogenous BAX (Fig. 4B) and BAK (fig. S5B). A similar result was observed when endogenous BAX was immunoprecipitated, and the presence of IRE1α-HA was assessed (fig. S5C). These interactions appeared to be specific because IRE1α-HA showed little or no association with BCL-2 (fig. S5D), BCL-$x_L$ (fig. S5E), or PERK (fig. S5F). The IRE1α-HA and BAX interaction required the cytosolic C-terminal region of IRE1α, which encodes the kinase and endoribonuclease domains (Fig. 4B). The interaction of BAX with endogenous IRE1α was also detected (Fig. 4C). Interestingly, the association of BAX with IRE1α was significantly increased in cells undergoing ER stress, which suggests that BAX preferentially interacts with active IRE1α (Fig. 4C).

BH3 and BH1 domains of BAK control its proapoptotic activity through interaction with other BCL-2–protein partners (*28*). Analysis of point mutants in the BH3 and BH1 domains (BAKmBH1, W122A, G123E, R124A; and BAKmBH3, L75E) (*29*, *30*), showed that these domains were also required for binding of BAK to IRE1α-HA (Fig. 4D). These same mutations also altered, to different extents, the ability of BAK to modulate IRE1α signaling (Fig. 4E). Taken together, these results suggest that the interaction between IRE1α and BAX and BAK is specific; requires the BH3 and BH1 domains; and, if disrupted, abolishes the effect of BAX/BAK on IRE1α signaling.

To assess whether impaired JNK phosphorylation and diminished XBP-1 expression indicated altered IRE1α activation in DKO cells, we examined the phosphorylation and dimerization status of IRE1α. DKO cells treated with brefeldin A for 2 to 4 hours did not show the mobility shift of IRE1α indicative of its autophosphorylation (Fig. 4F) (*8*). In addition, we detected the oligomerization of IRE1α in WT, but not in DKO, cells undergoing ER stress, by using nondenaturing gels or sedimentation in a sucrose gradient (fig. S6A and B). These data are consistent with diminished IRE1α signaling. Immunoprecipitation experiments revealed that more BiP was bound to IRE1α in DKO cells than in WT cells, under both basal and stress conditions (Fig. 4G), consistent with a decreased activation state of IRE1α in the DKO cells. Reconstitution of DKO cells with WT BAK or the ER-targeted mutant BAKcb5 decreased the basal binding of BiP to IRE1α, but expression of BAKmBH3 did not (Fig. 4H). Deletion of the cytosolic domain of IRE1α leads to basal binding of BiP that is not affected by BAX and BAK deficiency, which reinforces the idea that BAX and BAK regulate IRE1α activation through interaction with its cytosolic domain (fig. S6C). Finally, partial knockdown of BiP with small interfering RNA (siRNA) increased the rate of IRE1α activation in DKO cells as evidenced by increased expression of spliced XBP-1 (fig. S6D). Thus, BAX and BAK modulate IRE1α signaling through direct binding, possibly by the stabilization of the active form of the protein.

Our results reveal the proapoptotic proteins BAX and BAK as essential components of the UPR, a signaling system that allows secretory cells to handle the stress of protein folding, protein quality-control, and protein secretion. This function is independent of the proapoptotic function of BAX and BAK at the mitochondria and depends on the presence of the BH3-BH1 binding pocket. Under stress conditions, BAX and BAK interact with the cytosolic region of IRE1α, which is required for the modulation of IRE1α signaling (fig. S7). Members of the BCL-2 family are found in regulatory complexes at diverse organelles (*4*, *29*, *31*–*33*). Thus, members of the BCL-2 protein family may act as stress sentinels that connect stress signals to the proapoptotic core when cellular homeostasis is irreversibly altered.

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30. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. W122A, for example, represents the replacement of Ala with Trp at codon 122.
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**Supporting Online Material**

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# Natural Malaria Infection in *Anopheles gambiae* Is Regulated by a Single Genomic Control Region

Michelle M. Riehle,[1]* Kyriacos Markianos,[2]* Oumou Niaré,[3] Jiannong Xu,[1] Jun Li,[1] Abdoulaye M. Touré,[3] Belco Podiougou,[3] Frederick Oduol,[1] Sory Diawara,[3] Mouctar Diallo,[3] Boubacar Coulibaly,[3] Ahmed Ouatara,[3] Leonid Kruglyak,[4] Sékou F. Traoré,[3] Kenneth D. Vernick[1]†

We surveyed an *Anopheles gambiae* population in a West African malaria transmission zone for naturally occurring genetic loci that control mosquito infection with the human malaria parasite, *Plasmodium falciparum*. The strongest *Plasmodium* resistance loci cluster in a small region of chromosome 2L and each locus explains at least 89% of parasite-free mosquitoes in independent pedigrees. Together, the clustered loci form a genomic *Plasmodium*-resistance island that explains most of the genetic variation for malaria parasite infection of mosquitoes in nature. Among the candidate genes in this chromosome region, RNA interference knockdown assays confirm a role in *Plasmodium* resistance for *Anopheles Plasmodium-responsive leucine-rich repeat 1* (*APL1*), encoding a leucine-rich repeat protein that is similar to molecules involved in natural pathogen resistance mechanisms in plants and mammals.

The mosquito *Anopheles gambiae* is the major African vector of human malaria caused by *Plasmodium falciparum*. Genetically resistant and susceptible mosquitoes exist in nature, and that resistance can segregate as a simple Mendelian trait (*1*), but until now the prevalence, strength, and genomic location of such natural resistance loci in mosquitoes were not known. Genetic control of mosquito response to *Plasmodium* has been studied in an inbred laboratory strain of *A. gambiae* selected for the ability to melanize animal malaria parasites (*2*), for which *A. gambiae* is not a natural vector. However, associations uncovered in laboratory genetic mapping experiments may (*3*) or may not (*4*) hold when tested in nature.

We sampled genetic variation in a natural mosquito population by initiating isofemale pedigrees from wild mosquitoes captured in village dwellings in Mali, and we used the pedigrees to map allelic variants that have major effects on parasite development (*5*). Because female *A. gambiae* mate only once (*5*, *6*), each mosquito pedigree was the progeny of a single-pair cross that occurred in nature. Mosquito pedigrees were challenged by feeding on blood from natural human malaria infections in the same village. Thus, these experiments captured vector-parasite interactions of the evolutionary pair responsible for malaria transmission in the local population.

Each pedigree was fed malaria-infected blood from a single *P. falciparum* gametocyte carrier, and unfed mosquitoes were removed from the analysis. Thus, absence of oocysts resulted from failure of parasite development rather than lack of feeding. At 7 to 8 days after the infected blood meal, we dissected all mosquitoes and counted normal oocyst-stage parasites on the midgut. The number of oocysts constitutes the quantitative phenotype. If melanization of parasites was observed, which is an insect response to many pathogens (*2*), the fraction of melanized oocysts (melanized oocysts per total oocyst number) was also counted and used as a second, distinct quantitative phenotype. DNA extracted from each mosquito was genotyped with the use of 25 microsatellite markers spaced throughout the genome (*1*, *7*), enabling us to perform a genome-wide scan with ~9–centimorgan (cM) resolution. Linkage between DNA markers and either of the two quantitative phenotypes was detected with the use of nonparametric tests (*1*).

Of 101 pedigrees that were generated and challenged, 27 met criteria for genotyping: (i) >20 mosquitoes, and (ii) at least 30% of mosquitoes with normal oocysts, or any mosquitoes with melanized parasites (fig. S1). Notably, 22 pedigrees had no infected individuals despite feeding on blood that contained gametocytes. Such completely uninfected pedigrees are not suitable for genetic analysis, but their large number suggests that mosquito resistance to *P. falciparum* is common.

Among 17 genotyped pedigrees [including two from our previous work (*1*)], significant linkage between quantitative infection phenotype and DNA marker(s) was detected in seven pedigrees [genome-wide $P$ value < 0.05 by permutation (*5*)]: five loci linked to *P. falciparum* infection intensity (Pfin) and two linked to *P. falciparum* melanization (Pfmel) (Fig. 1 and Table 1). Overall, 41% of pedigrees analyzed revealed a significant locus, indicating that loci with a major effect on parasite development in the field population are widespread. All analyzed pedigrees were independently generated from wild isofemales. Each named locus was the only significant locus found in the pedigree. Secondary loci with influence on *Plasmodium* may segregate in some of these pedigrees, but if so, none had a strong enough influence on parasite development to be independently identified as a distinct locus with genome-wide significance.

Although our experiments detect significant linkage to loci on all three chromosomes, the loci with the strongest effect colocalize in a small region of chromosome arm 2L (Fig. 1 and Table 1). Four of the seven independent loci mapped to a 15–megabase (Mb) region of chro-

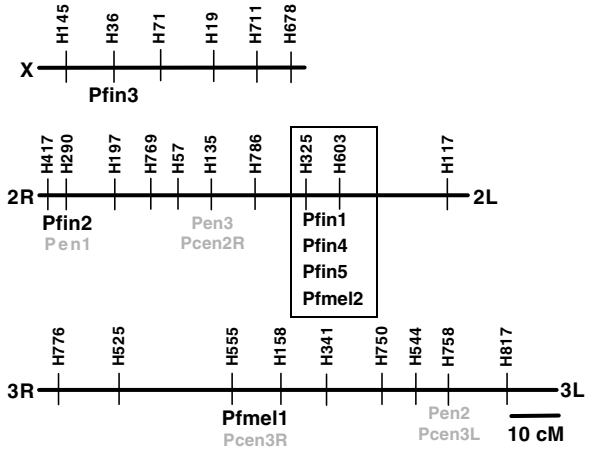


**Fig. 1.** Genome-wide *A. gambiae* linkage map. Genomic location of loci linked with Pfin (number of normal oocysts following infected blood meal) or Pfmel (fraction of melanized parasites) in wild isofemale pedigrees from Mali, West Africa. Each locus shows significant linkage in a single F1- or F2-generation pedigree to indicated marker locus. Horizontal lines indicate chromosomes (R, right arm; L, left arm), and vertical lines indicate microsatellite markers. Naturally occurring *P. falciparum* resistance loci are shown in black. Pen and Pcen loci (gray) indicate the genomic location of melanization loci mapped in a laboratory-selected *Plasmodium*-melanizing line of *A. gambiae* challenged with two different strains of the simian model parasite, *P. cynomolgi* (Pen, *P. cynomolgi* B; Pcen, *P. cynomolgi* Ceylon) (*15*, *16*). The box highlights the genomic cluster of loci with linkage to *P. falciparum* infection outcome in four independent mosquito pedigrees.

[1]Center for Microbial and Plant Genomics and Department of Microbiology, University of Minnesota, St. Paul, MN 55108, USA. [2]Program in Computational Biology, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, M2-B876, Seattle, WA 98109, USA. [3]Département d'Epidémiologie des Affectations Parasitaires, Université de Bamako, Boîte Postale 1805, Bamako, Mali. [4]Lewis-Sigler Institute for Integrative Genomics and Department of Ecology and Evolutionary Biology, Carl Icahn Laboratory, Princeton University, Princeton, NJ 08544, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: kvernick@umn.edu

mosome 2L, 5 Mb on either side of markers H603 and H325 [$P \leq 0.014$ for clustering of loci (*5*)]. For fine mapping of the clustered loci, six to ten additional markers (*5*) were tested in the four pedigrees (Fig. 2). Three of the four clustered loci (Pfin1, Pfin4, and Pfin5) stand out among all mapped loci, because they have by far the lowest *P* values and the greatest influence on parasite numbers (Table 1). For all three Pfin loci, the presence of a resistance allele explains at least 89% of parasite-free mosquitoes within a pedigree; the fourth locus in the cluster, Pfmel2, explains 100% of mosquitoes with melanized parasites within the pedigree. The four clustered loci display dominant or semidominant effects on parasite infection. In contrast, Pfin2 and Pfin3, located outside this cluster, display recessive inheritance of resistance and explain little of the variation in infection phenotype. Given the data, linkage in this region could be due to four distinct loci in the population, different allelic forms of the same locus, or a single causative mutation. Regardless, a specific chromosome region, which controls the majority of naturally segregating variation for *P. falciparum* infection detected in this study, is a major *Plasmodium*-resistance island (PRI) of the *A. gambiae* genome. Finding linkage in independent pedigrees over multiple years, with each pedigree fed on infected blood from a different gametocyte carrier, further suggests that the resistance effect controlled by the mosquito PRI is largely independent of parasite genotype.

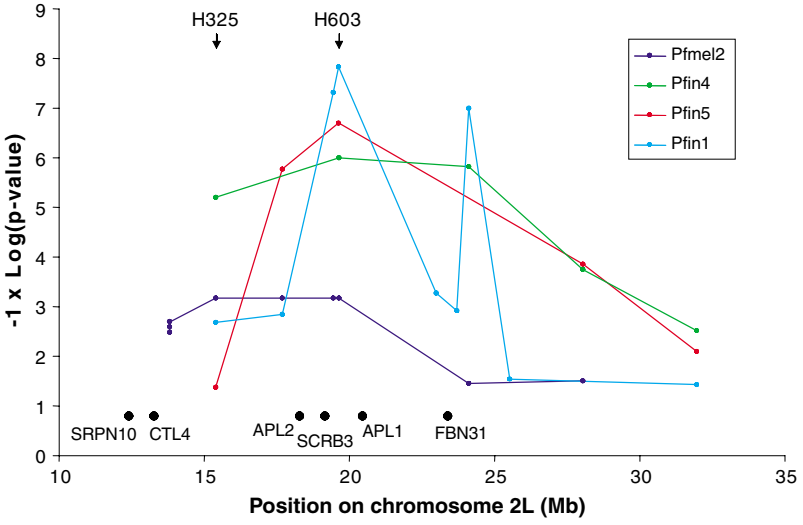


**Fig. 2.** Fine mapping of the *Plasmodium* resistance island. Nominal *P* values are shown as a function of position for the four pedigrees defining the PRI. The map shows consistent evidence of significant linkage over closely spaced microsatellite markers (points on graphed lines) in all four pedigrees with significant linkage to two distinct infection phenotypes, oocyst intensity (Pfin) and parasite melanization (Pfmel) (See (*5*) for markers). The locations of the markers yielding significant linkage signal in the initial 9-cM scan, H325 and H603, are indicated by arrows. Only the most informative markers are shown (≥3 genotypes in a pedigree). For clarity, the labeled candidate genes above the *x* axis are those annotated immune genes that were also either transcriptionally regulated after *P. berghei* infection or present in an immune-enriched EST library. Predicted functions or functional domains: SRPN10, serpin; CTL4, c-type lectin; SCRB3, scavenger receptor; and FBN31, fibrinogen. All candidate genes are shown in fig. S2.

The small size of the PRI makes feasible a search for candidate genes. Three filters were applied to the 976 Ensembl-predicted transcripts in this 15-Mb interval (Fig. 3 and fig. S2) (*5*). First, the interval contained 39 predicted members of mosquito immune gene families by sequence annotation, including putative functions in pathogen recognition, immune signal modulation, immune effectors, and signal transduction. Second, 13 genes in the interval were transcriptionally regulated on whole-genome microarrays after infection with *P. berghei*. Third, 29 genes were represented in an enriched *A. gambiae* immune expressed sequence tag (EST) library containing a wide spectrum of host defense genes, including transcriptional response to *Plasmodium*. Taking into account overlaps between categories, the filters highlight 72 genes. Despite caveats of the filters (e.g., causative genes might not belong to known families of immune genes or be transcriptionally regulated by *Plasmodium* infection), these genes represent a plausible set of candidate genes for initial screening.

Genes for two novel leucine-rich repeat (LRR)–containing proteins (Ensembl *A. gambiae* genome database accession codes ENSANGG00000012041 and ENSANGG00000019333) were the only ones to satisfy all filter criteria (Fig. 3). These genes are named *Anopheles Plasmodium-responsive leucine-rich repeat 1* (*APL1*) and *APL2* (respective to the accession codes above). Interestingly, the 39 immune genes by annotation in the PRI included 13 genes for LRR proteins, among them APL1 and APL2, and the numerically largest genomic cluster of LRR genes coincides with the PRI (fig. S3). LRR proteins have known roles in innate immunity in many phyla as direct or indirect pathogen recognition factors such as NACHT-LRR proteins, Toll-like receptors, and plant R genes (*8*–*10*). Plant R genes form genomic clusters of rapidly evolving homologs (*11*).

**Table 1.** Pedigrees with significant linkage. We examined two *P. falciparum* resistance phenotypes in *A. gambiae* 8 days after a malaria-infected blood meal: (i) normal parasite count and (ii) rate of parasite melanization. Nominal *P* values are derived from two-tailed Wilcoxon-Mann-Whitney tests, whereas genome-wide *P* values result from $10^5$ to $10^8$ simulations per pedigree, as appropriate. Neither of the pedigrees yielding Pfmel loci gave Pfin loci, nor did the Pfin4 pedigree give a Pfmel locus. Gen, generation after wild mother. *N*, number of females in the pedigree. Prevalence, percentage of mosquitoes in pedigree with at least one normal oocyst per midgut. Melanization rate, fraction of total malaria parasites melanized in individuals with at least one parasite melanized. Allele effect, dominant or codominant inheritance; genotype effect, recessive inheritance.

| Locus | Location | Gen | *N* | Prevalence (%) | Oocyst range | | Melanization rate (%) | *P* value | | Effect |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Normal | Melanized | | Nominal | Genome-wide | |
| Pfin1 | 2L | F2 | 83 | 67 | 0–116 | 0 | 0 | $1.47 \times 10^{-8}$ | $1.20 \times 10^{-7}$ | Allele |
| Pfin2 | 2R | F2 | 82 | 96 | 0–66 | 0 | 0 | $7.56 \times 10^{-4}$ | $2.66 \times 10^{-2}$ | Genotype |
| Pfin3 | X | F2 | 152 | 72 | 0–31 | 0 | 0 | $1.12 \times 10^{-4}$ | $2.59 \times 10^{-3}$ | Genotype |
| Pfin4 | 2L | F1 | 45 | 80 | 0–69 | 0–51‡ | 46 (24–91) | $9.78 \times 10^{-7}$ | $3.02 \times 10^{-6}$ | Allele |
| Pfin5 | 2L | F1 | 62 | 53 | 0–37 | 0 | 0 | $1.37 \times 10^{-7}$ | $5.60 \times 10^{-7}$ | Allele |
| Pfmel1 | 3R | F1 | 40 | 100* | 4–151 | 0–117 | 39 (2–85) | $1.84 \times 10^{-4}$ | $4.98 \times 10^{-3}$ | Allele |
| Pfmel2 | 2L | F1 | 21 | 100† | 1–87 | 0–43 | 60 (31–77) | $2.30 \times 10^{-4}$ | $1.35 \times 10^{-3}$ | Allele |

*Melanization prevalence (fraction of individuals in pedigree with at least one melanized parasite) is 42.5%. †Melanization prevalence is 29%. ‡Melanization prevalence is 22%.

The roles of APL1 and APL2 were functionally tested by RNA interference (RNAi)–mediated reductions of gene expression in laboratory colony mosquitoes infected with *P. berghei* (*5*). APL1 knockdown mosquitoes carried significantly higher numbers of oocysts (by a factor of 6 to 15, $P < 0.007$) than did controls treated with green fluorescent protein (GFP) double-stranded RNA (Fig. 4 and fig. S4). Few or no melanized parasites were observed in the APL1 and APL2 knockdown mosquitoes, similar to controls. In contrast, reduction of APL2 did not affect oocyst number ($P = 0.515$). Thus, APL1 mediates significant protection from *Plasmodium* infection and could underlie, at least in part, the phenotypic effect of the PRI. However, APL1 is only a candidate that must await further genetic and functional studies for confirmation of a role in natural transmission. In this regard, the phenotypes in the APL1 knockdowns and controls (high versus moderate oocyst number) do not precisely reproduce those observed in susceptible and resistant Pfin genotypes after natural infection (many versus zero oocysts). Exact replication of the field phenotype may require specific aspects of the genetic background found in field mosquitoes or the specific APL1 allele present in the natural pedigrees. Also, response to *P. berghei* may be different from response to *P. falciparum*. Resolving the exact role, if any, of APL1 in the natural transmission system will require additional fieldwork.

There is no previous information on genetic control of malaria parasite melanization in nature. Our study shows that control of melanization maps to two different loci, Pfmel1 and Pfmel2. Melanization was inefficient as a resistance mechanism because parasite removal by melanization was only partial, as compared with complete elimination by the Pfin loci. Moreover, pedigrees segregating Pfmel alleles displayed 100% prevalence of normal parasites despite the ability to melanize (Table 1). The particularly high prevalence of normal parasites could indicate that natural melanization alleles, while killing the melanized parasites, may also cause defects in other mechanisms that otherwise limit parasite numbers. On balance, the observed melanizing genotypes would likely result in more, not less, efficient transmission of *Plasmodium* in nature. Given the data from these pedigrees, melanization of parasites in *A. gambiae* is not a major component of natural *Plasmodium* resistance.

We report a comprehensive population survey of *A. gambiae* in Mali using a study design that allows identification and genetic mapping of loci with major effect on *P. falciparum* development in the field population. We detected genetic loci responsible for two distinct outcomes of *Plasmodium* infection, variation in numbers of normal parasites and rate of parasite killing by melanization. Most loci with major effect were clustered, forming a significant PRI in the *A. gambiae* genome. Annotation information and laboratory-based experiments were used to extract candidate genes for functional testing.

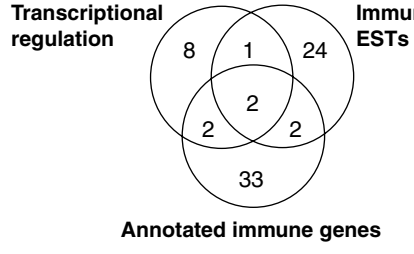


**Fig. 3.** Candidate gene filtering. Venn diagram of candidate genes in the 15-Mb interval surrounding the PRI, 5-Mb outside markers H325 and H603 [nucleotide coordinates 2L:10398172 to 24623620 on the 2La inverted chromosome (*5*)]. Venn segments indicate numbers of genes with changes in transcript abundance 24 hours after *P. berghei* infection by whole-genome microarray (false discovery rate = 0.05), predicted immune genes by annotation, and genes represented by ESTs in a subtracted immune-enriched library (*5*). Intersection of the three filtering criteria within the PRI contains the two predicted LRR-containing proteins APL1 and APL2.

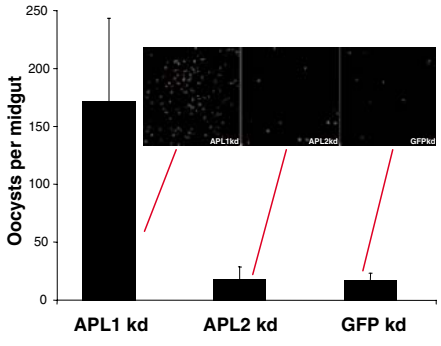


**Fig. 4.** Silencing of APL1 gene expression increases *Plasmodium* infection. APL1 significantly limits the efficiency of malaria parasite infection in four independent knockdown experiments. APL1 kd, oocyst numbers in mosquitoes 7 to 8 days after RNAi-mediated reduction of APL1 transcript abundance followed by infection with *P. berghei*; APL2 kd, mosquitoes treated with APL2 double-stranded RNA; GFP kd, control mosquitoes treated with GFP double-stranded RNA. The APL1 and GFP data are averages of the mean oocyst number from four independent experiments, and error bars indicate standard error. APL2 data comes from three replicate experiments that tested APL2 along with APL1 and GFP control. The APL1 kd results in oocyst loads 6 to 15 times as high as those of GFP controls ($P < 0.007$). The APL1 kd effect was significant by Wilcoxon-Mann-Whitney, Student's *t* test, and Kolmogorov-Smirnov when analyzed for pooled data across replicates or in analysis of individual replicates. The APL2 kd had no significant effect on parasite number ($P = 0.515$). (Inset) Fluorescence micrographs of oocysts [constitutively fluorescent line PbGFPCON (*17*)] in midguts of APL1 kd, APL2 kd, and GFP kd mosquitoes, respectively.

Examination of malaria transmission in wild populations is essential to understand and ideally capitalize on the efficient malaria conrol strategies already implemented by *A. gambiae* in nature. For example, one possible malaria control approach would increase the population frequency of pre-existing natural resistance alleles like those described here by elevating the fitness cost of malaria parasite infection (*12*). Recently reported entomopathogenic fungi can disproportionately kill *Plasmodium*-infected mosquitoes as compared with uninfected ones (*13*, *14*), and thus could have the properties required of a selective agent to transform vector populations to *Plasmodium* resistance. Such an evolutionary engineering strategy would not require introduction of new genetic information into natural vector populations.

The most notable feature of the observed mosquito resistance is that it segregates as a simple Mendelian trait of major effect at reasonably high frequency in randomly sampled natural genotypes. Interestingly, many mosquito pedigrees completely eliminated the parasite despite feeding on infected blood. We speculate that the "wild-type" mosquito phenotype is resistance and that susceptibility should be attributed to specific points of failure or loss of function in the mosquito immune system.

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### Supporting Online Material

www.sciencemag.org/cgi/content/full/312/5773/577/DC1
Materials and Methods
Figs. S1 to S4
References

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# Autophagic Fungal Cell Death Is Necessary for Infection by the Rice Blast Fungus

**Claire Veneault-Fourrey, Madhumita Barooah, Martin Egan, Gavin Wakley, Nicholas J. Talbot***

Rice blast is caused by the fungus *Magnaporthe grisea*, which elaborates specialized infection cells called appressoria to penetrate the tough outer cuticle of the rice plant *Oryza sativa*. We found that the formation of an appressorium required, sequentially, the completion of mitosis, nuclear migration, and death of the conidium (fungal spore) from which the infection originated. Genetic intervention during mitosis prevented both appressorium development and conidium death. Impairment of autophagy, by the targeted mutation of the *MgATG8* gene, arrested conidial cell death but rendered the fungus nonpathogenic. Thus, the initiation of rice blast requires autophagic cell death of the conidium.

Rice blast disease is one of the most serious and recurrent problems affecting rice production worldwide (*1*–*4*). The disease is caused by *Magnaporthe grisea* (Hebert) Barr (anamorph *Pyricularia grisea* Sacc), an ascomycete fungus that produces dome-shaped melanin-pigmented appressoria that penetrate the leaves and stems of rice plants (*Oryza sativa*). Rice blast disease starts when three-celled spores land on the leaf surface, to which they stick tightly by means of an adhesive carried in the spore apex (*5*, *6*). Within an hour after bonding to the leaf surface, each spore germinates and produces a polarized germ tube, which typically emerges from the apical cell of the conidium (77.7 ± 5.8% of cases). Thereafter, the germ tube quickly differentiates at its tip to form an appressorium. The *M. grisea* appressorium generates up to 8 MPa of turgor to provide the motive force necessary to breach the tough cuticle of the rice plant via a narrow penetration peg (*7*, *8*). After storage products are transported from the spore to the developing appressorium, the conidium and germ tube then collapse and die (movie S1).

To investigate the genetic control of appressorium morphogenesis and to observe the pattern of nuclear division during appressorium development in *M. grisea*, we used a strain of the fungus [expressing a histone H3–enhanced green fluorescent protein (eGFP) fusion protein (*9*)] in which individual nuclei were visible by epifluorescence microscopy (Fig. 1). During spore germination on an in-

School of Biosciences, University of Exeter, Washington Singer Laboratories, Perry Road, Exeter EX4 4QG, UK.

*To whom correspondence should be addressed. E-mail: N.J.Talbot@exeter.ac.uk

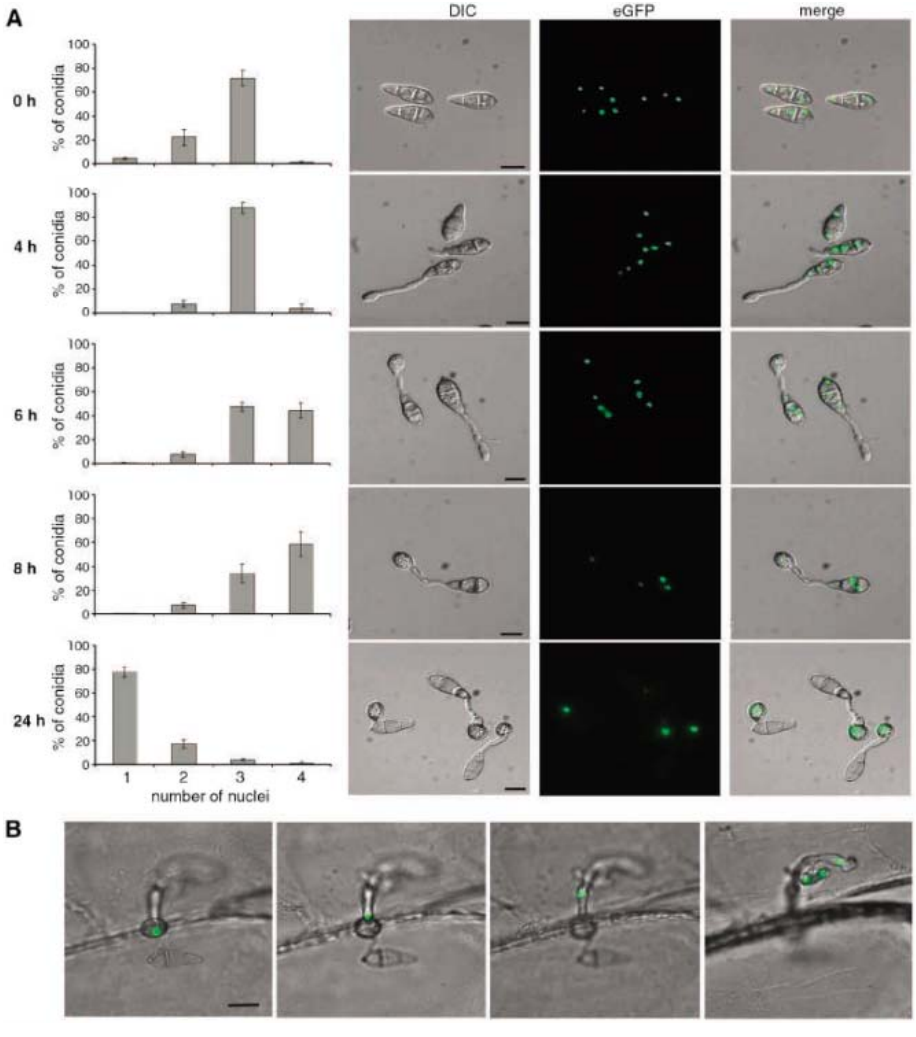


**Fig. 1.** (**A**) Bar charts showing the number of *M. grisea* germlings containing 0 to 4 nuclei (mean ± SD; $n$ = 200; three experiments). Conidia expressing *H3:eGFP* were examined by epifluorescence microscopy at 0, 2, 4, 6, 8, and 24 hpi. Representative bright-field [differential interference contrast (DIC)], fluorescence, and merged images at each time point are shown. (**B**) Merged bright-field and epifluorescence images from a Z-stack experiment using sterile onion epidermal strips inoculated with conidia expressing *H3:eGFP* and observed at 30 hpi. Similar observations were made using rice leaves. Scale bars, 10 μm.

ductive hydrophobic surface, a fungal nucleus migrated into the germ tube, where mitosis occurred between 4 and 6 hours after inoculation (hpi, hours post inoculation) (Fig. 1A). After 8 hpi, one of the daughter nuclei migrated into the incipient appressorium, and the other nucleus returned to the conidium. After 12 to 15 hpi, the three nuclei that remained in the conidium broke down and could no longer be seen (Fig. 1A). By 24 hpi, the only surviving nucleus was contained within the mature appressorium. To determine whether the pattern of nuclear division and migration was associated specifically with appressorium morphogenesis, we conducted two experiments. First, we incubated conidia in the presence of exogenous nutrients, which inhibits appressorium formation in *M. grisea* (*4*) and leads to the germination of nondifferentiated fungal hyphae. Under these conditions, mitosis occurred later (6 to 8 hours) and nuclei in the conidium did not degenerate (fig. S1, A and B). Second, we observed mitosis in a *M. grisea Δpmk1* mitogen-activated protein kinase mutant which is unable to form appressoria (*10*). The *Δpmk1* mutant underwent successive nuclear divisions within the germ tube as it extended, but no break down of nuclei in the spore took place (fig. S1C). Thus, in *M. grisea*, (i) mitosis consistently occurred within the germ tube before appressorium differentiation, and (ii) breakdown of nuclei within the conidium was correlated with appressorium development. After plant infection, the appressorial nucleus migrated to the penetration hypha and underwent mitosis before the development of the invasive hypha (Fig. 1B).

Next, we tested whether the formation of the appressorium was a prerequisite for infection-related development in *M. grisea*. We used two drugs, hydroxyurea (HU) and benomyl, to block cell-cycle progression at the $G_1$/S or $G_2$/M transitions, respectively (*11*). When HU (50 mM) or benomyl (20 μg $ml^{-1}$) was applied to conidia between 0 and 4 hpi, inhibition of mitosis was observed in the germ tube (fig. S2, A and B); at 24 hpi, appressorium development had declined significantly ($P < 0.05$) (fig. S2C). However, when HU or benomyl was applied later (at 6 or 8 hpi), mitosis had already taken place and the rate of appressorium differentiation was not significantly reduced ($P > 0.05$). Initiation of appressorium development, characterized by hooking of the apex of the germ tube, still occurred after benomyl treatment, but cells failed to mature (fig. S2D).

To investigate the importance of mitotic control to appressorium development in *M. grisea*, we generated a mutant that was unable to enter mitosis. In *Aspergillus nidulans*, the *NimA* gene (*never entry into mitosis*) encodes a protein kinase that is necessary for mitosis, in addition to the $p34^{cdc2}$/cyclin B–dependent kinase (*12*, *13*). We identified an equivalent gene in *M. grisea*, *MgNIMA* (fig. S3A), which was able to complement a *nimA5* mutant of *A. nidulans* (fig. S3D). We then generated a thermosensitive *MgnimA$^{E37G}$* mutant allele (*14*) and introduced this into the *M. grisea H3:eGFP* strain by targeted gene replacement (fig. S3, B and C). *MgnimA$^{E37G}$* mutants grew normally at 26°C but showed a reversible growth defect at 32°C (Fig. 2A). To investigate how the *MgnimA$^{E37G}$* mutation affects mitosis in *M. grisea*, we counted the number of nuclei contained in conidia incubated at various temperatures (from 26° to 32°C) during a time course of appressorium development. When incubated at 26°C, conidia from the *H3:eGFP* strain and the *MgnimA$^{E37G}$* mutant followed the same progression through mitosis, which had normally occurred by 6 hpi (Fig. 2, B to D). Mitosis was not affected when conidia of the *H3:eGFP M. grisea* strain were incubated at 29°C (Fig. 2, B to D). In contrast, when conidia of the *MgnimA$^{E37G}$* strain were shifted to 29°C, they most commonly displayed

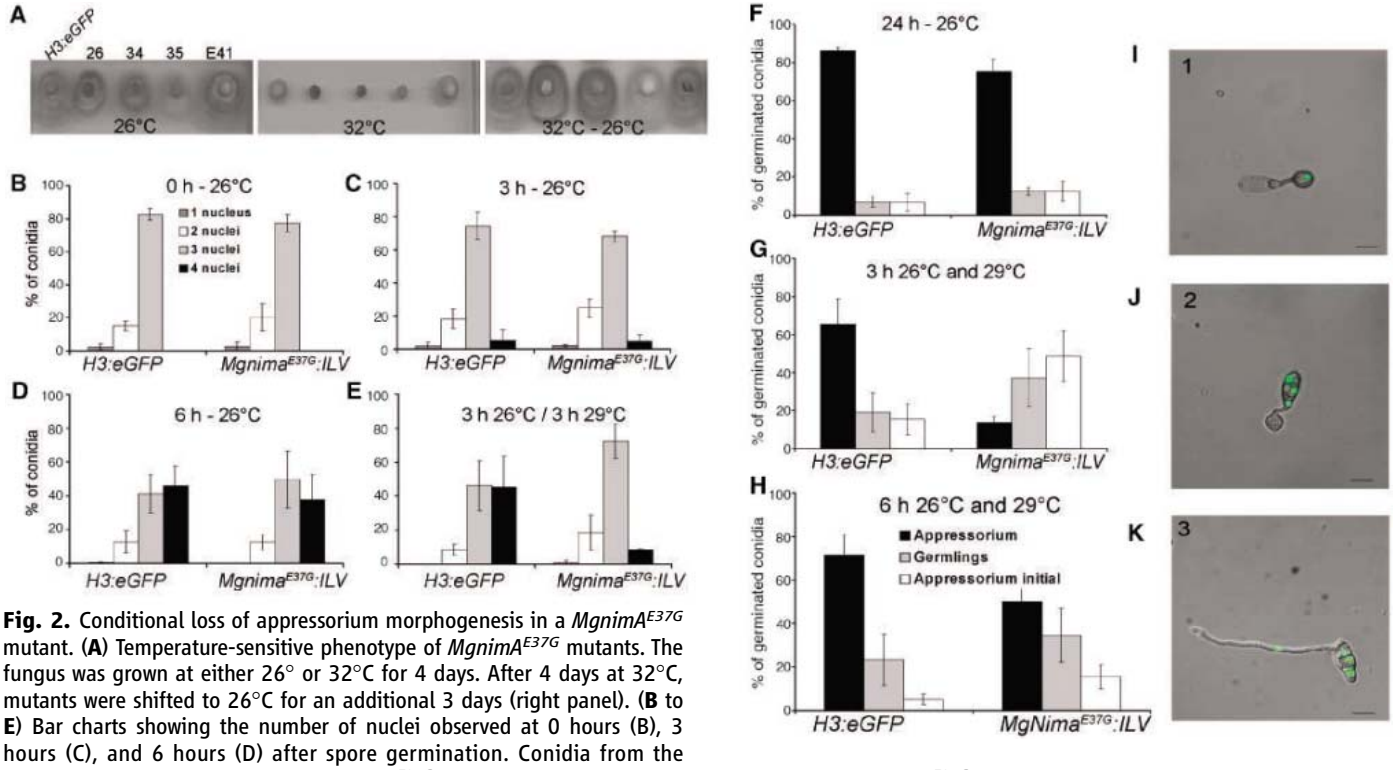


**Fig. 2.** Conditional loss of appressorium morphogenesis in a *MgnimA$^{E37G}$* mutant. (**A**) Temperature-sensitive phenotype of *MgnimA$^{E37G}$* mutants. The fungus was grown at either 26° or 32°C for 4 days. After 4 days at 32°C, mutants were shifted to 26°C for an additional 3 days (right panel). (**B** to **E**) Bar charts showing the number of nuclei observed at 0 hours (B), 3 hours (C), and 6 hours (D) after spore germination. Conidia from the *M. grisea H3:eGFP* strain and *MgnimA$^{E37G}$* mutant were allowed to germinate and form appressoria at 26°C. After 3 hours at 26°C, slides were transferred to 29°C, a semi-permissive temperature that does not interfere with appressorium development (E), and the number of nuclei was recorded 3 hours later (mean ± SD, $n = 200$, three experiments). (**F** to **H**) Bar charts showing frequency of appressorium formation. Conidia from the *H3:eGFP* strain and the *MgnimA$^{E37G}$* mutant were allowed to form appressoria at 26°C (F). After 3 hours (G) or 6 hours (H) at 26°C, slides were transferred to 29°C. Appressorium differentiation was recorded after 24 hours (mean ± SD, $n = 200$). (**I** to **K**) Micrographs showing nucleated mature appressorium (I), appressorial initial (J), and undifferentiated germling (K). Scale bars, 10 μm.

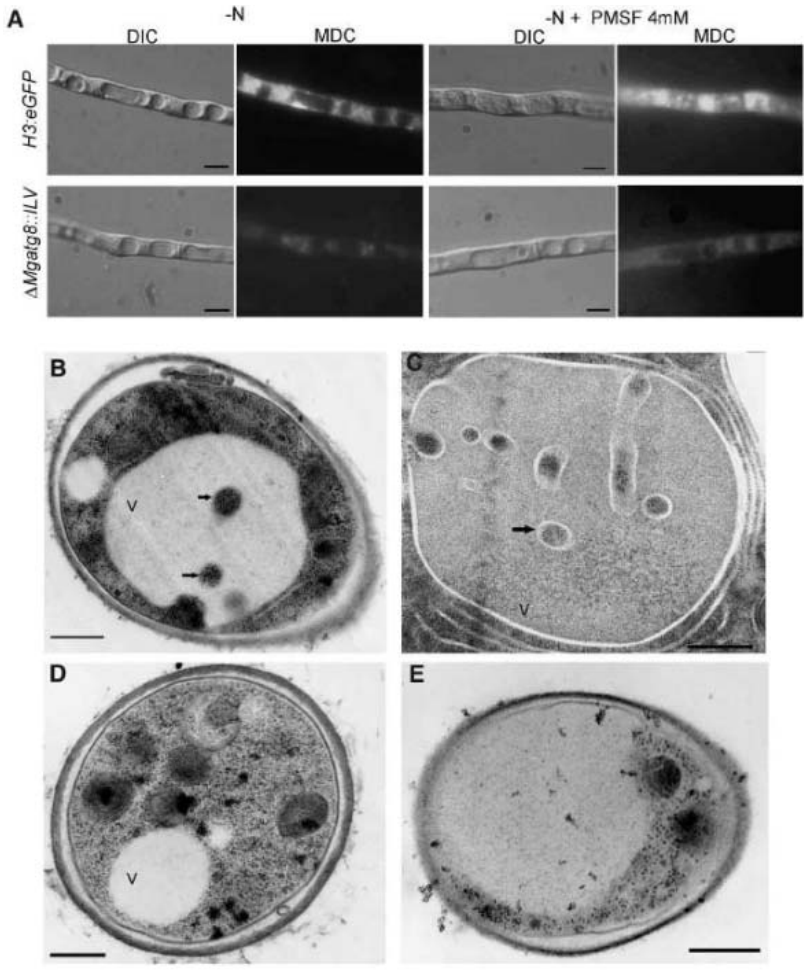


**Fig. 3.** *MgATG8* is necessary for autophagy in *M. grisea*. (**A**) Micrographs showing hyphae of *M. grisea H3:eGFP* and *ΔMgatg8::ILV* strains subjected to nitrogen starvation, in the presence or absence of 4 mM PMSF. DIC and epifluorescence images of MDC staining (*10*) are shown. The granular appearance of vacuoles corresponds to the accumulation of autophagic bodies in the hyphae of the *H3:eGFP* strain. Scale bars, 10 μm. (**B** to **E**) Representative electron micrographs of vacuoles (v) in hyphae of a *M. grisea* wild-type Guy11 strain [(B) and (C)] or a *ΔMgatg8* mutant [(D) and (E)] subjected to nitrogen starvation in the presence of PMSF. Arrows indicate autophagy-associated structures. Scale bars, 500 nm.

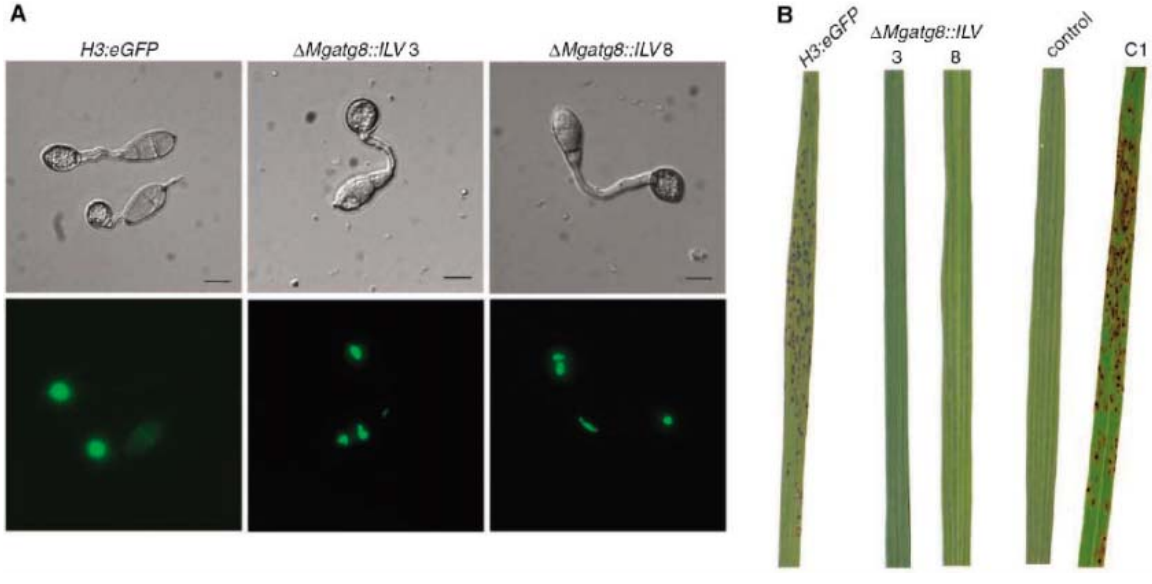


**Fig. 4.** Disruption of autophagy in *M. grisea* prevents rice blast disease. (**A**) Micrographs showing appressorium development and nuclei in *H3:eGFP* and two *ΔMgatg8::ILV* mutants. Scale bars, 10 μm. (**B**) *ΔMgatg8::ILV* mutants are unable to cause rice blast disease. Seedlings of rice cultivar CO-39 were inoculated with uniform conidial suspensions of *H3:eGFP* or *ΔMgatg8::ILV* mutants 3 and 8. Seedlings were incubated for 5 days to allow development of disease symptoms. Reintroduction of the *MgATG8* gene restored the ability to cause rice blast disease to a *ΔMgatg8* mutant (C1). "Control" represents a mock inoculation with 0.2% gelatin.

three nuclei, demonstrating that mitosis had not taken place (Fig. 2E). The small number of germlings with four nuclei had probably completed mitosis before the shift in temperature.

To determine the effects of arresting mitotic progression on the formation of appressoria in the *H3:eGFP* strain and *MgnimA*$^{E37G}$ mutants, we incubated conidia at 26°C and then transferred them to 29°C after 3 hours (before mitosis) or 6 hours (after mitosis). Three types of development were distinguished: mature melanin-pigmented appressoria, undifferentiated

germlings, or appressorial initials showing swelling of the germ tube apex (Fig. 2, I to K). No significant differences ($P > 0.05$) were observed in conidia from the wild-type strain and the *MgnimA*$^{E37G}$ mutant maintained at 26°C (Fig. 2F) or when conidia were shifted to 29°C after 6 hours (Fig. 2H). However, the rate of appressorium differentiation declined significantly ($P < 0.01$) between the *MgnimA*$^{E37G}$ mutant and the wild-type strain when conidia were shifted to 29°C before mitosis had occurred (3 hpi) (Fig. 2G). Thus, pharmacological and genetic interventions in mitotic progression both prevent the formation of the infection structure in *M. grisea*. Furthermore, blocking mitosis and appressorium development also prevented the collapse and death of the conidium.

Why is appressorium morphogenesis in *M. grisea* always accompanied by collapse of the spore and nuclear degeneration (Fig. 1B)? These processes might be a consequence of autophagy occurring within the spore after mitosis and nuclear migration. To test this idea, we identified the *M. grisea MgATG8* gene, which is very similar to the *ATG8/AUT7* gene of *Saccharomyces cerevisiae* (*15*), and were able to restore autophagy when the gene was expressed in a *ΔMgatg8* mutant of *S. cerevisiae* (fig. S4). We generated *ΔMgatg8* mutants in both Guy11 and *H3:eGFP* genetic backgrounds (*11*) and tested their ability to undergo autophagy in response to nitrogen starvation (Fig. 3A). When hyphae were starved in the presence of an inhibitor of vacuolar serine proteases [phenylmethylsulfonyl fluoride (PMSF) (*16*)], autophagic bodies accumulated in the vacuoles of the *H3:eGFP M. grisea* strain, leading to a granular appearance of the vacuole (Fig. 3A). In contrast, vacuoles of the *ΔMgatg8::ILV* strains displayed normal morphology without granulation, indicating that autophagy did not occur in the mutant. We also used mono-dansyl cadaverine (MDC) as an indicator of autophagic activity (*16*, *17*), which displayed strong fluorescence in the cytoplasm and vacuoles of the *H3:eGFP* strain, but little fluorescence in the starved mycelium of the *ΔMgatg8::ILV* mutant (Fig. 3A). Electron microscopy showed autophagic bodies (*15*) inside the vacuoles of the wild-type strain (Fig. 3, B and C), which were absent in a *ΔMgatg8::ILV* mutant (Fig. 3, D and E). *MgATG8* is therefore necessary for autophagy in *M. grisea*.

To investigate the effects of inhibiting autophagy during the development of appressoria in *M. grisea*, we incubated conidia on a hard hydrophobic surface to develop appressoria. In the wild-type strain, appressoria formed after 24 hours and the collapse of the conidium was accompanied by nuclear degeneration (Fig. 4A). Conversely, conidia of the *ΔMgatg8::ILV* mutants still formed appressoria, but three misshapen and elongated nuclei were present in spores at 24 hpi and conidia from the *ΔMgatg8::ILV* mutants were still intact (Fig. 4A). However, the *ΔMgatg8* mutants were unable to carry out plant infection (Fig. 4B) or form penetration hyphae efficiently even after 48 hpi (table S1). We verified that the different mutant phenotypes attributed to the *MgATG8* deletion, including an effect on the efficiency of conidiogenesis (table S1), were complemented by transforming the *ΔMgatg8::hph* strain with a wild-type copy of *MgATG8* under the control of its native promoter (*11*) (Fig. 4A).

We have demonstrated a correlation among mitotic control, autophagic cell death, and infection-related morphogenesis of a pathogenic fungus. Mitosis is completed before the morphogenesis of the appressorium, suggesting that either a $G_2$/M or a postmitotic checkpoint may regulate appressorium formation in *M. grisea* (*18*). Autophagic cell death of the fungal spore is also coupled to mitotic completion, is a prerequisite for infection, and perhaps constitutes a further developmental checkpoint for the establishment of rice blast disease in *O. sativa*.

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### Supporting Online Material

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Movie S1

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# Global Control of Dimorphism and Virulence in Fungi

**Julie C. Nemecek,[1] Marcel Wüthrich,[2] Bruce S. Klein[1,2,3,4]***

**Microbial pathogens that normally inhabit our environment can adapt to thrive inside mammalian hosts. There are six dimorphic fungi that cause disease worldwide, which switch from nonpathogenic molds in soil to pathogenic yeast after spores are inhaled and exposed to elevated temperature. Mechanisms that regulate this switch remain obscure. We show that a hybrid histidine kinase senses host signals and triggers the transition from mold to yeast. The kinase also regulates cell-wall integrity, sporulation, and expression of virulence genes in vivo. This global regulator shapes how dimorphic fungal pathogens adapt to the mammalian host, which has broad implications for treating and preventing systemic fungal disease.**

Microbial pathogens that inhabit our environment must undergo a radical change to survive inside a mammalian host. Among the more than 100,000 different species of environmental fungi are six phylogenetically related ascomycetes called the dimorphic fungi: *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Sporothrix schenkii*, and *Penicillium marneffei.* These fungi change morphology once spores are inhaled into the lungs of a mammalian host from hyphal molds in the environment to pathogenic yeast

[1]Department of Medical Microbiology and Immunology, [2]Department of Pediatrics, [3]Department of Internal Medicine, and [4]The Comprehensive Cancer Center, University of Wisconsin Medical School, University of Wisconsin Hospital and Clinics, Madison, WI 53792, USA.

*To whom correspondence should be addressed. E-mail: bsklein@wisc.edu

forms. Dimorphic fungi inhabit the soil worldwide and collectively cause over a million new infections a year in the United States alone. They tend to remain latent after infection and may reactivate if the subject becomes immune deficient (*1*–*5*). It has long been believed that phase transition from mold to yeast is obligatory for pathogenicity, but the mechanism that regulates this switch has remained a mystery. In this report, we provide firm genetic evidence that establishes the central role of dimorphism in pathogenicity, and we describe a regulator of this morphologic transition.

It is temperature that induces dimorphic fungi to change phases (*6*). At 25°C, they grow as mold. At 37°C, the core temperature of humans, they switch into the pathogenic yeast form (*7*), during which yeast phase–specific virulence genes are induced. Few of these genes have been identified; among the best studied are *BAD1* of *B. dermatitidis*, *CBP1* of *H. capsulatum*, and the 1,3-α-glucan synthase (*AGS1*) of these fungi and of *P. brasiliensis* (*8*–*10*). We postulated that deciphering the regulation of phase-specific genes would elucidate the control of morphogenesis.

Forward genetics, a process of inducing mutations randomly in a genome to detect phenotypes and linked genes, has advanced our understanding of microbial pathogenesis. Dimorphic fungi have not yet been manipulated in this way because the classical genetic approaches have proved too cumbersome, and the molecular tools have been unavailable. We previously showed that *Agrobacterium tumefaciens* transfers DNA randomly into the genomes of *B. dermatitidis* and *H. capsulatum*, primarily into single sites and without recombination, which, in theory, allows the identification of recessive mutations (*11*). Here, we used *A. tumefaciens* for insertional mutagenesis in a dimorphic fungus to attempt to uncover regulators of yeast phase–specific genes and phase transition from mold to yeast.

*BAD1* of *B. dermatitidis* was used as "bait" in hunting for regulators of dimorphism, because it is expressed during the transition to yeast, regulated transcriptionally, and required for pathogenicity (*12*, *13*). To identify mutants with regulatory defects, we created a *B. dermatitidis* reporter strain T53-19 harboring a transcriptional fusion between the *BAD1* promoter and β-galactosidase reporter, $P_{BAD1}$-*LacZ*. We transformed T53-19 with *A. tumefaciens* and monitored regulatory defects using a color screen (*14*). As yeast at 37°C, the reporter strain stains blue on media containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal). As mold at 22°C, it fails to stain and is white. Because *BAD1* expression is up-regulated in yeast, we

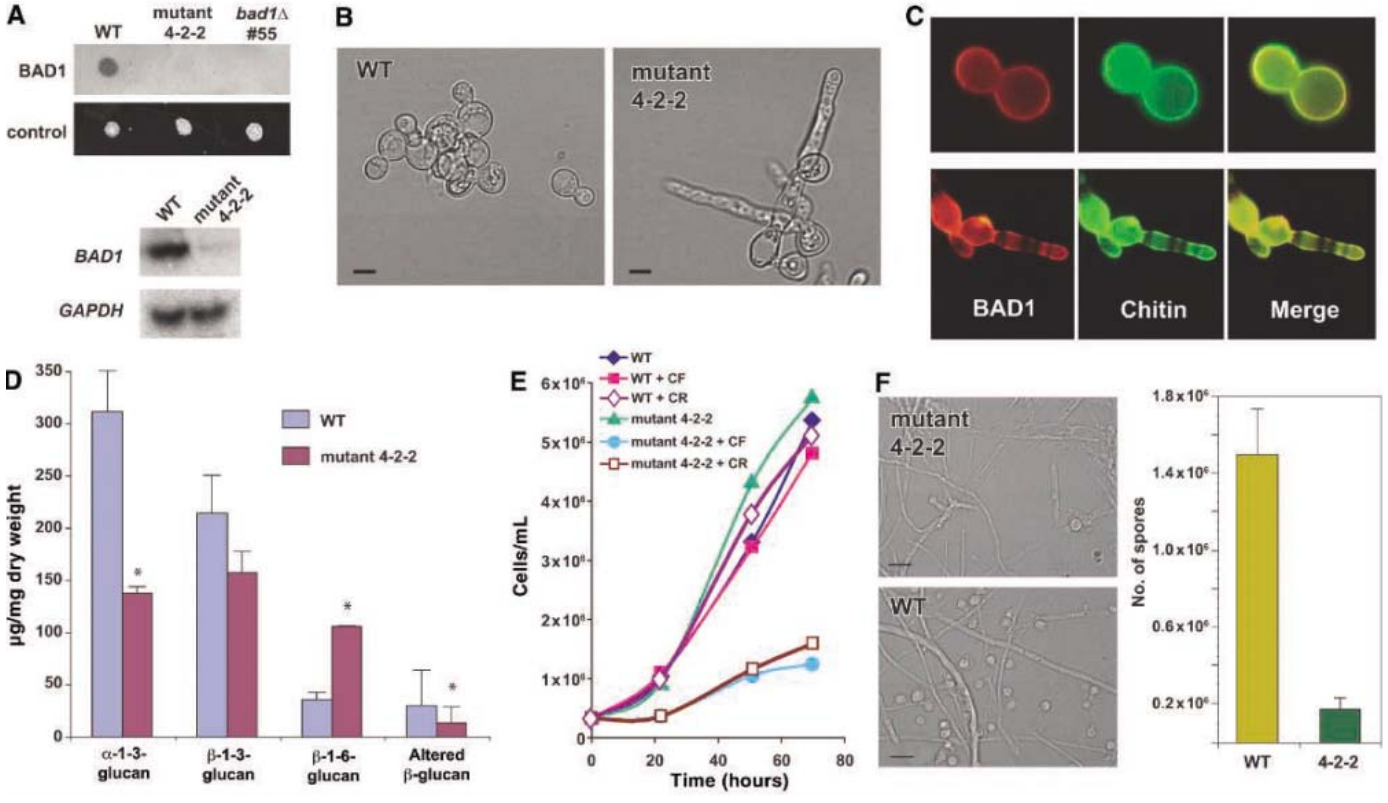


**Fig. 1.** An insertional mutant of *B. dermatitidis* with pleiotropic defects in morphogenesis, virulence gene expression, cell wall integrity, and sporulation. (**A**) (Top) Fungal colony overlay and immunoblot for BAD1. Nitrocellulose overlay of colonies probed with DD5-CB4 monoclonal antibody (mAb) to BAD1. Parental reporter strain T53-19 (WT) is a positive control and *bad1*Δ strain 55, a negative control. The patches of fungal cells tested are shown below the blot. (Bottom) Northern analysis. By densitometry, *BAD1* transcript in the mutant is 14% of that in the wild type. *GAPDH*, loading control. (**B**) Microscopic appearance of mutant and wild-type parent strain grown at 37°C in liquid *Histoplasma* macrophage medium (HMM). The mutant phenotype is stable on serial passage. Scale bar, 10 μm. (**C**) Surface BAD1 (red), chitin (green), and merged images. (Top) Wild type; (bottom) mutant. BAD1 is stained with mAb DD5-CB4 and goat antibody to mouse phycoerythrin, and chitin with wheat germ agglutinin–fluorescein isothiocyanate. (**D**) Cell wall composition. Cell walls were obtained and analyzed as described (*14*). The mutant has half as much 1,3-α-glucan and three times as much 1,6-β-glucan as the wild-type strain. *$P < 0.05$, ANOVA test. Data are means ± SD of three experiments. (**E**) Sensitivity to the cell wall–binding agents calcofluor (CF) and Congo red (CR). Growth at 37°C of wild-type reporter strain and mutant was analyzed in liquid HMM alone or with CF or CR (20 μg/ml). Cells were counted to quantify growth rate. Data from one representative experiment of three are shown. Results were similar at 22°C (fig. S1B). (**F**) Sporulation of mold. (Left) Sporulating hyphae of the wild-type and mutant strains on potato flake agar. (Right) Total number of spores produced after 2 weeks' growth at 22°C (*14*). Data are representative of two experiments. Scale bar, 10 μm.

sought mutants that were white instead of blue at 37°C. In screening ~15,000 transformants, we found seven with color defects. These transformants were confirmed by colony immunoblot and Northern analysis to have diminished BAD1 and reductions in transcript from <1% to 14% of the reporter strain (*14*).

One mutant, 4-2-2, had a reduction in *BAD1* transcript to 14% of the reporter strain, corresponding reduction in BAD1 (Fig. 1A), and pleiotropic defects. At 37°C, 4-2-2 fails

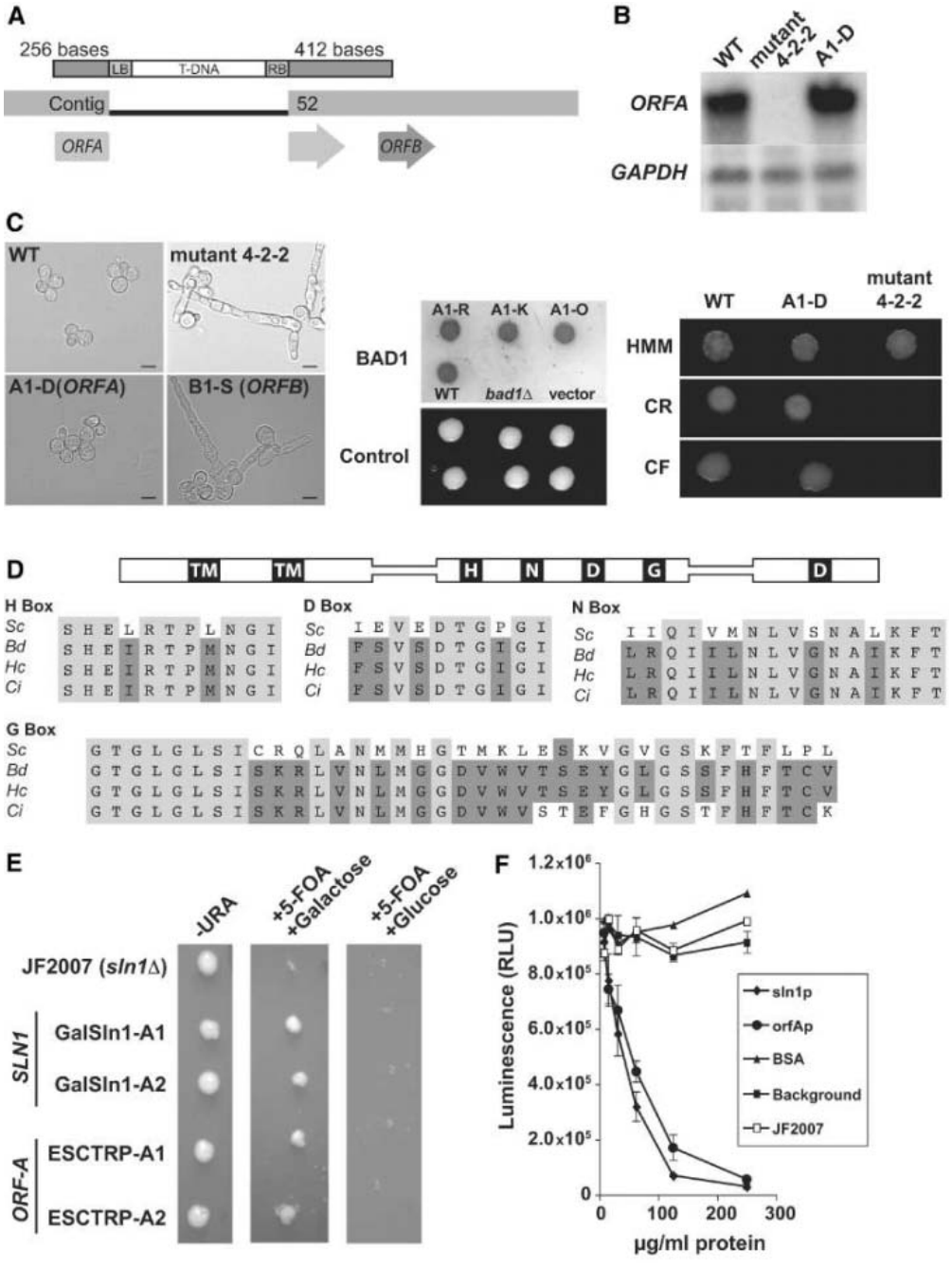


**Fig. 2.** Elucidation of the genotype of mutant 4-2-2. (**A**) A single T-DNA insertion is present in the mutant. Adapter PCR identified 256 bases on the left border and 412 bases on the right. Flanks have homology to contig 52 in the *Blastomyces* genome. Putative ORFs (A and B) are located near the insertion. T-DNA was inserted into the 3825-nucleotide ORFA coding sequence 522 nucleotides upstream of the stop codon. The insertion interrupts the first β sheet of the protein's receiver domain (see Fig. 2D). ORFB is 1.4 kb away from the T-DNA insertion. LB denotes the T-DNA left border, and RB the right border. (**B**) Northern analysis of ORFA transcript. A1-D is a transformant of mutant 4-2-2 complemented with an intact genomic copy of ORFA and flanking sequence. (**C**) ORFA complements the defects in 4-2-2. (Left) ORFA restores yeast morphology to mutant 4-2-2, whereas ORFB does not. (Middle) Fungal colony immunoblot for BAD1. In transformants re-expressing ORFA (A1-R, A1-K, A1-O), BAD-1 is detectable. Negative controls are *bad1*Δ strain 55 and a transformant of strain 4-2-2 that received a control vector lacking ORFA ("vector"). The patches of fungal cells tested are shown below the blot. (Right) Growth of mutant, wild type, and a complemented strain A1-D on HMM in the presence of calcofluor (CF) or Congo red (CR) (20 μg/ml). Top line shows growth of the strains on HMM alone. Scale bar, 10 μm. (**D**) ORFA has the domain structure and sequence of histidine kinase and is conserved in dimorphic fungi. ORFA has a histidine-containing H-box, an aspartate-containing D-box, and G- and N-boxes (*32*). Two putative transmembrane domains (TM) and an aspartate-containing receiver domain at the C terminus are also present. Sequences homologous to the *S. cerevisiae* (*Sc*) histidine kinase *SLN1* and *B. dermatitidis* (*Bd*) histidine kinase are present in other dimorphic fungi *H. capsulatum* (*Hc*) and *C. immitis* (*Ci*). (**E**) *Blastomyces* ORFA complements an *sln1* defect in *S. cerevisiae*. *S. cerevisiae* JF2007 [*sln1*::*LEU2*, *ura3-52*, *trp1*Δ63, *his3*Δ200, *leu2*Δ1, *lys2*, pRSPTP2 (*URA3*)] was transformed with a galactose-inducible vector containing a c-Myc–tagged *SLN1* (pGalSln1-A1 and A2) or a FLAG-tagged ORFA (pESCTRP-422-A1 and A2). Both vectors contained *TRP1* for selection. Transformants were initially plated on medium lacking uracil, to select for pRSPTP2, and lacking tryptophan, to select for the expression vector. Transformants were then plated on medium containing 5-FOA to select against pRSPTP2 and containing galactose for induction. pRSPTP2 rescues the lethal *sln1* defect. Only transformants with a functional histidine kinase that complements the *snl1* defect can grow on 5-FOA media under inducing conditions (*14*, *20*). Transformants were plated on 5-FOA–containing medium with glucose as a control for gene induction. (**F**) Kinase activity detected by a luminescence assay. Decreasing relative light units (RLUs) indicate increasing kinase activity. Protein was immunoprecipitated from *S. cerevisiae* JF2007 transformed with c-Myc–tagged *SLN1* expression vector (Sln1p), FLAG-tagged ORFA expression vector (orfAp), or untransformed JF2007 (JF2007), by using Myc-specific or FLAG-specific antibody (*14*). Bovine serum albumin (BSA) and reaction buffer (background) are negative controls. Data are the means ± SD of three experiments.

to convert to yeast and grows as pseudohyphae (Fig. 1B). Cell wall composition is altered in the mutant (Fig. 1, C and D). BAD1 and chitin are distributed in an aberrant striated pattern in the cell wall, and the amount of 1,3-α-glucan is greatly reduced. As evidenced in other cell-wall mutants (*15*), mutant 4-2-2 is more sensitive than the parent strain to the cell wall–binding chemicals calcofluor and Congo red (Fig. 1E). After sporulation, mutant 4-2-2 produces nearly 10% as many conidia (the infectious particles) as the parental strain (Fig. 1F), and spore progeny of the mutant retain a pseudohyphal phenotype when grown at 37°C (fig. S1A). Despite these pleiotropic defects, the mutant grows at the same rate as the parent strain (table S1) (*14*). Mutant 4-2-2 thus has global defects in morphogenesis, cell wall composition, sporulation, and expression of virulence factors BAD1 and 1,3-α-glucan.

By Southern analysis, we determined that transferred DNA (T-DNA) had inserted into one site in the genome of mutant 4-2-2. By adapter polymerase chain reaction (PCR), 256 and 412 nucleotides flanking 5′ and 3′ of the T-DNA insertion, respectively, were amplified and sequenced. Flanking sequence showed identity to contig 52 in the *Blastomyces* genome (*16*). Two large putative open reading frames (ORFs) (ORFA and ORFB) were identified near the insertion site (Fig. 2A). The T-DNA transected ORFA, whose transcript is not detectable in mutant 4-2-2 by Northern analysis (Fig. 2B). To assess the functional role of these ORFs in mutant 4-2-2, we complemented the strain with a gene copy of ORFA or ORFB (*14*) (Fig. 2B). A plasmid containing an intact genomic copy of ORFA and flanking sequence reversed the phenotypic defects in the mutant (Fig. 2C), whereas that containing ORFB and flanking sequence did not.

ORFA encodes a protein of 1274 residues (on the basis of transcript size) and predicted by gene-finding software (Softberry, Mount Kisco, NY). The gene has three exons totaling 3825 base pairs (bp) and two introns of 140 and 40 bp, and it displays homology to domains of histidine kinase by BLAST analysis and CD search. Histidine kinases are signal transduction proteins that organisms in all three domains of life use to respond to environmental signals (*17*) and control developmental processes (*18*, *19*). ORFA is predicted to have two transmembrane domains and the necessary elements for histidine kinase function, including the histidine-containing H-box and aspartate-containing D-box involved in phosphorelay (Fig. 2D). The sequence also contains the N- and G-boxes used in ATP-binding and catalytic function, and an aspartate-containing receiver domain. The *B. dermatitidis* sequence is homologous to the hybrid histidine kinase *SLN1* in *Saccharomyces cerevisiae* and to sequences in the genomes of *H. capsulatum* and *C. immitis*, dimorphic fungi for which extensive genome sequence is available (Fig. 2D).

We assayed the histidine kinase activity of ORFA using genetic and biochemical approaches. The ORFA of *B. dermatitidis* was expressed heterologously in *S. cerevisiae* to see if it functionally complements a *sln1* defect in strain JF2007 (*20*). *S. cerevisiae* has a single hybrid histidine kinase, Sln1p, which regulates an osmosensing mitogen-activated protein kinase (MAPK) cascade, an oxidative stress-response pathway, and cell wall biosynthesis (*21*, *22*). The lethal *sln1* defect in JF2007 is viable because of the presence of a plasmid containing the phosphatase gene *PTP2*. Ptp2p dephosphorylates the Hog1 protein that accumulates in the absence of the functional histidine kinase (*23*). After lithium acetate transformation of JF2007 with an expression vector containing either ORFA or *SLN1*, we selected against maintenance of the *PTP2* transgene by examining growth on 5-fluoroorotic acid (5-FOA). Transformants receiving either *SLN1* or ORFA survived the loss of PTP2, which implies that ORFA functionally complements the *sln1* defect (Fig. 2E and fig. S2). In biochemical studies, the *B. dermatitidis* ORFA protein product, immunoprecipitated from *S. cerevisiae* transformants, exhibited histidine kinase activity similar to that of Sln1p in a luminescence assay (Fig. 2F). ORFA thus encodes a protein that functions genetically and biochemically as a histidine kinase.

To test the role of *B. dermatitidis* histidine kinase unambiguously in the global defects observed in mutant 4-2-2, we created a targeted knockout by allelic replacement (*14*) (fig. S3A). The knockout is locked in the mold form at 37°C (Fig. 3A) and has all of the pleiotropic defects of mutant 4-2-2 [impaired BAD1 and 1,3-α-glucan expression, sensitivity to calcofluor and Congo red,

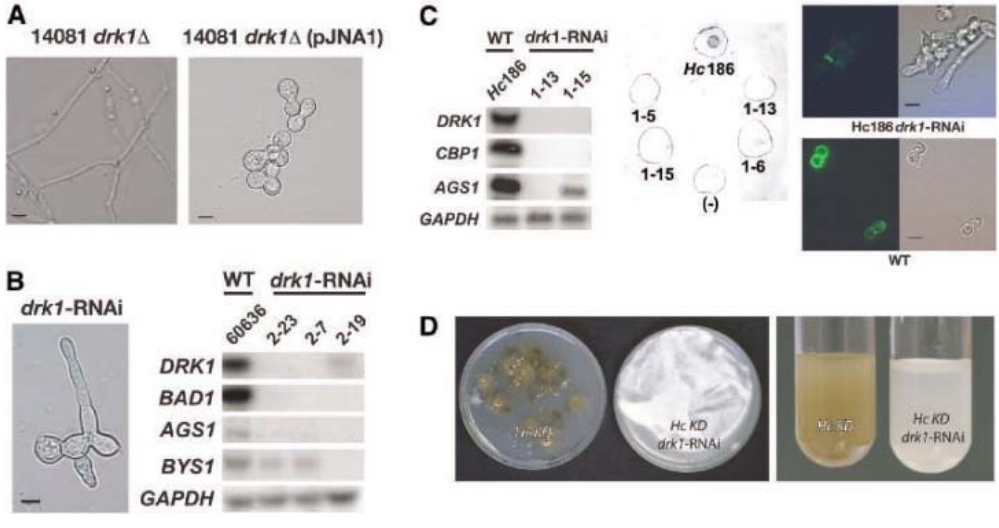


**Fig. 3.** The histidine kinase *DRK1* regulates dimorphism from mold to yeast and virulence gene expression in *B. dermatitidis* and *H. capsulatum*. (**A**) (Left) Knockout strain (14081 *drk1*Δ) grown at 37°C is locked in the mold morphology. (Right) Complemented strain 14081 *drk1*Δ (pJNA1) regains the parental yeast phenotype at 37°C. Scale bar, 10 μm. (**B**) (Left) Gene silencing of *DRK1* by RNAi in *B. dermatitidis* 60636 (*drk1*-RNAi) induces pseudohyphal morphology at 37°C. Scale bar, 10 μm. (Right) Northern analysis of virulence factors *BAD1* and *AGS1* and yeast phase-specific gene *BYS1* in three independent *DRK1*-silenced transformants of *B. dermatitidis* parental strain 60636. *GAPDH*, loading control. (**C**) (Left panel) Northern analysis of two independent *DRK1*-silenced tranformants of *H. capsulatum* strain 186AR *ura5*, probing for the expression of *DRK1* and virulence genes *CBP1* and *AGS1*. (Middle) Ruthenium red stain of CBP in culture supernatant. Four independent *DRK1*-silenced transformants of *H. capsulatum* (1-5, 1-13, 1-15, and 1-6) show decreased staining compatible with reduced CBP. Parental strain 186AR *ura5* is a positive control, and medium (-) a negative control. (Right) Surface 1,3-α-glucan in a *DRK1*-silenced strain of *H. capsulatum* (Hc186 *drk1*-RNAi) and parental 186AR *ura5* (WT). Cells were stained with mAb MOPC104e and goat antibody to mouse fluorescein isothiocyanate (*14*). Light image is on the right. Scale bar, 10 μm. (**D**) Pigmentation of *H. capsulatum* wild-type (*Hc*KD) and *DRK1*-silenced strain (*Hc*KD *drk1*-RNAi). Mold on agar plates (left) and a suspension of the harvested spores (right).

and failure to sporulate] to a more extreme extent (fig. S3, B to D). Complementing the knockout corrected these defects (Fig. 3A and fig. S3). Henceforth, we refer to the gene here as *DRK1* for dimorphism-regulating histidine kinase. We were unable to test virulence of *DRK1* knockout strains in mice because the hyphae could not be reliably quantified and no spores were made. The more severe phenotype of the knockout compared with the insertion mutant 4-2-2 suggests that there is residual gene activity in the latter, perhaps due to the partial function of a truncated protein or to minimal *DRK1* transcript beneath the level of detection.

We exploited RNA interference (RNAi) for gene silencing in *B. dermatitidis* to knock down *DRK1* function and to circumvent the extreme phenotypes of the knockout (*14*). RNAi experiments were carried out in two different *B. dermatitidis* strains: 60636 and 14081. *DRK1*-silenced transformants from both strains exhibit rough colony morphology and pseudohyphal growth at 37°C, reduced sporulation, and sensitivity to calcofluor and Congo red (Fig. 3B; and fig. S4 and table S2). To explore the relation between *B. dermatitidis DRK1* and expression of yeast-phase virulence genes, we analyzed transcript for *DRK1*, *BAD1*, and *AGS1* by Northern analysis in *DRK1*-silenced strains. *BAD1* and *AGS1* transcripts are absent in parallel with that of *DRK1*, and the transcript for *BYS1*, a yeast-phase gene of unknown function, is inconsistently reduced in the strains (Fig. 3B).

The *DRK1* sequence and its key domains are highly conserved in *H. capsulatum* and *C. immitis* (Fig. 2D). To test whether *DRK1* acts as a global regulator of dimorphism and yeast-phase virulence gene expression in other dimorphic fungi, we used RNAi to silence gene expression in *H. capsulatum*. *DRK1* was silenced in strain 186*ura5*AR and a clinical isolate, HcKD (*14*). Transformants were initially screened for those that grew as pseudohyphae at 37°C. *DRK1*-silenced strains showed concomitant reduction in the expression of virulence factors CBP and 1,3-α-glucan (*9*, *24*), sensitivity to calcofluor, and reduced sporulation (Fig. 3C; and fig. S5 and table S2). Transcript levels for the silenced strains were correspondingly reduced for *DRK1*, *CBP1*, and *AGS1* (Fig. 3C). Strikingly, the brown pigment indicative of melanin in mycelia and spores of wild-type *Histoplasma* strains was absent in the *DRK1*-silenced

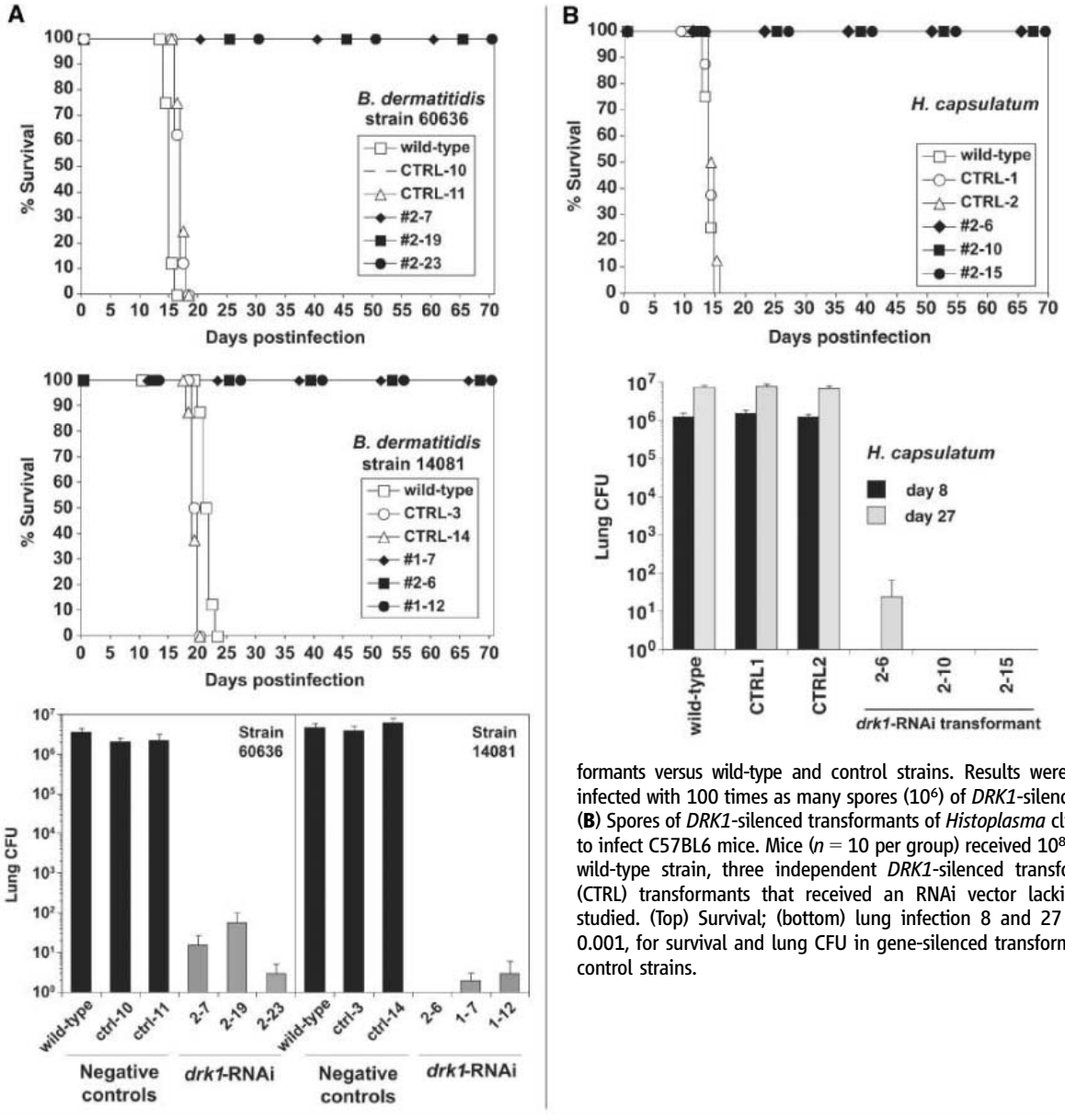


**Fig. 4.** Effect of knocking down *DRK1* expression on the in vivo pathogenicity of *B. dermatitidis* and *H. capsulatum* in murine models of pulmonary infection. (**A**) Spores of *DRK1*-silenced transformants from *Blastomyces* strains 60636 and 14081 were used to infect C57BL6 mice. Mice ($n = 10$ per group) received $10^4$ spores intratracheally. The wild-type strain, three independent *DRK1*-silenced transformants, and two control (CTRL) transformants that received an RNAi vector lacking the target sequence were studied. (Top and middle) Survival; (bottom) burden of lung infection (CFUs) in mice 14 days after infection. $P < 0.001$, for survival and lung CFU in the gene-silenced transformants versus wild-type and control strains. Results were similar when mice were infected with 100 times as many spores ($10^6$) of *DRK1*-silenced transformants (fig. S6). (**B**) Spores of *DRK1*-silenced transformants of *Histoplasma* clinical isolate KD were used to infect C57BL6 mice. Mice ($n = 10$ per group) received $10^8$ spores intratracheally. The wild-type strain, three independent *DRK1*-silenced transformants, and two control (CTRL) transformants that received an RNAi vector lacking target sequence were studied. (Top) Survival; (bottom) lung infection 8 and 27 days after infection. $P < 0.001$, for survival and lung CFU in gene-silenced transformants versus wild-type and control strains.

strains (Fig. 3D). Melanin is linked with virulence in other fungal pathogens, including *C. neoformans* and *A. fumigatus* (*25*). The histidine kinase *DRK1* thus regulates global functions, including dimorphism and virulence gene expression in *H. capsulatum*.

Because mold-to-yeast transition and expression of the yeast-phase genes *BAD1*, *CBP1*, and *AGS1* are required for pathogenicity, we postulated that silencing *DRK1* expression would impair virulence of *B. dermatitidis* and *H. capsulatum.* We investigated virulence of *DRK1*-silenced strains in a mouse model of lethal pulmonary infection (*14*). After intratracheal infection with spores of *B. dermatitidis*, *DRK1*-silenced strains from two independent isolates were sharply attenuated compared with wild-type strains, as measured by survival and lung colony-forming units (CFUs) (Fig. 4A). In a murine model of histoplasmosis after intratracheal infection with spores, *DRK1*-silenced strains of *H. capsulatum* also were sharply reduced in virulence compared with wild-type strains (Fig. 4B). The growth rate of *DRK1*-silenced strains in all genetic backgrounds was similar to that of the respective parent strain (table S1). Silencing expression of the histidine kinase *DRK1*, therefore, reduced pathogenicity markedly in two dimorphic fungi.

We have described a highly conserved hybrid histidine kinase, *DRK1*, that is indispensable for dimorphism, virulence gene expression, and pathogenicity in dimorphic fungi. Our finding that *DRK1* gene disruption locks a dimorphic fungus in the mold form uncovers a long-sought regulator of phase transition. The observation that phase-locked cells lose virulence extends the biochemical studies of Medoff *et al*. (*7*) and offers genetic proof that conversion of mold to yeast is required for pathogenicity in dimorphic fungi. A change in shape alone probably does not explain why the conversion is required, because mold and yeast differ in the expression of many genes and phenotypes, including some that are linked with virulence.

Two-component signaling systems are widespread in the prokaryotes. Eukaryotes have been thought to rely mainly on serine, threonine, and tyrosine kinases for signal transduction, but histidine kinase two-component systems have recently been shown to play a role in environmental sensing and cell development in eukaryotes (*26*), for example, in *Candida albicans*, where they regulate filamentation (*18*, *19*). We show that a histidine kinase regulates sensing of environmental changes needed for mold-to-yeast transition in at least two dimorphic fungal pathogens. Histidine kinase homologs were identified in three dimorphic species for which the most complete genome sequence is available: *B. dermatitidis*, *H. capsulatum*, and *C. immitis*. The presence of this gene in multiple species and its conserved role in *B. dermatitidis* and *H. capsulatum* suggests that it may control phase transition and virulence gene expression, as well as cell wall development and sporulation, in the other systemic dimorphic fungi. *DRK1* shares limited sequence similarity with histidine kinases that regulate filamentation in the more distantly related fungus *C. albicans*, although the functional domains are conserved. Nevertheless, the finding that histidine kinases regulate changes in shape for diverse fungal species points to a potentially broad role of these environmental sensors in the fungal kingdom.

What is the environmental signal that *DRK1* of *Blastomyces* and *Histoplasma* senses to regulate phase transition and virulence gene expression? In *S. cerevisiae*, Sln1p detects osmotic stress, whereas in *Schizosaccharomyces pombe*, the histidine kinase–regulated *SPC1* MAPK cascade senses osmotic stress as well as oxidative and heat stress and nutrient deprivation (*27*). Potential signals for histidine kinase sensing in dimorphic fungi include temperature, osmotic or oxidative stress, nutrient deprivation, redox potential, and host-derived factors such as hormones like 17-β-estradiol, which induces germ tubes in *C. albicans* (*28*) and blocks mold-to-yeast transition of *P. brasiliensis* (*29*).

In *S. cerevisiae*, the hybrid histidine kinase Sln1p transfers a phosphoryl group to the histidine residue of the phosphotransfer (HPt) domain in Ypd1p (*30*). Ypd1p transfers a phosphoryl group to one of two response regulators, Ssk1p or Skn7p, which control MAPK cascades and gene expression. Ypd1p, Ssk1p, and Skn7p homologs are present in both the *Blastomyces* and *Histoplasma* genomes; three other putative histidine kinases also are present (*16*). The four histidine kinases may sense different environmental signals that all lead through Ypd1p to the same output of morphogenesis and virulence gene expression. Alternatively, multiple downstream response regulators could respond to stimulation from Ypd1p, each controlling a distinct program involved in phase transition.

Histidine kinases linked with two-component relays have been identified in all three domains of life, but none have been established in any of the fully sequenced vertebrate genomes. The lack of such a homolog in humans suggests that these proteins may serve as antifungal drug targets. Previously identified bacterial histidine-kinase inhibitors have had general antifungal activity that is not kinase-specific; instead, it results in general membrane damage (*31*). Greater knowledge of eukaryotic histidine kinase function could assist in the development of better-targeted inhibitory compounds. Dimorphic fungi attenuated by knocking out histidine kinase might also be used for vaccination purposes.

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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/312/5773/583/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 and S2
References and Notes

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# A Voltage Sensor–Domain Protein Is a Voltage-Gated Proton Channel

Mari Sasaki,[1,2,3] Masahiro Takagi,[1,3] Yasushi Okamura[1,2,3,4]*

Voltage-gated proton channels have been widely observed but have not been identified at a molecular level. Here we report that a four-transmembrane protein similar to the voltage-sensor domain of voltage-gated ion channels is a voltage-gated proton channel. Cells overexpressing this protein showed depolarization-induced outward currents accompanied by tail currents. Current reversal occured at equilibrium potentials for protons. The currents exhibited pH-dependent gating and zinc ion sensitivity, two features which are characteristic of voltage-gated proton channels. Responses of voltage dependence to sequence changes suggest that mouse voltage-sensor domain–only protein is itself a channel, rather than a regulator of another channel protein.

Voltage-gated ion channels are composed of six transmembrane segments (S1 to S6). S5 and S6 form the hydrophilic pore, while S1 to S4 constitute the voltage-sensor domain (VSD) (*1*, *2*). S4 has positively charged amino acids that are periodically aligned at every third position, and these are known to be essential for sensing the change in membrane potential (*1*). Amino acid substitution in S4 can confer ion-conducting activity to the VSD (*3*–*6*). We recently identified a voltage-sensor–containing phosphatase protein (VSP) that contains a VSD-like domain and a phosphatase domain (*7*). The VSD-like domain was shown to regulate enzymatic activity of the phosphatase domain (*7*). Thus, VSD domains may have functions beyond voltage sensing and may be distributed more widely than only in channel proteins.

We used the amino acid sequence of the VSD of *Ciona intestinalis* VSP (Ci-VSP) (*7*) as a query for searching with Washington University Basic Local Alignment Search Tool (WUBLAST) software (*8*) to identify a mouse cDNA, registered as RIKEN cDNA 0610039P13 in the GenBank database. The encoded protein consists of four transmembrane segments with overall homology to the VSD, but it lacks any structure corresponding to the pore domain of voltage-gated channels (fig. S1). We thus named it mVSOP (mouse voltage-sensor domain–only protein). Ortholog genes were found in the genomes of ascidians, zebrafish, *Xenopus*, and mammals. The putative S4 segment of the deduced protein shows alignment of the positively charged residues similar to that conserved in conventional voltage-gated channels (fig. S1). The putative S2 and S3 segments contain negatively charged residues (fig. S1). These charge distributions are conserved among all ortholog proteins.

Based on the similarity of mVSOP to the VSD of voltage-gated channels, we tested whether it exhibits gating currents. We transfected tsA201 cells, which are derivatives of HEK293 cells, with mVSOP cDNA. We used whole-cell patch clamping and did not observe any trace of gating currents. Instead, robust voltage-dependent outward currents were elicited (Fig. 1), despite the apparent lack of the pore domain. These currents were activated slowly during the depolarizing pulse, and activation became faster as membrane potentials became more positive (Fig. 1A). The average maximum amplitude was 60 ± 38 pA/pF ($n$ = 16 cells) for a 100-mV pulse applied for 500 ms. Inward tail currents were observed during repolarization, indicating that mVSOP is not acting as a pump. Untransfected tsA201 cells showed almost no outward or inward current under the same conditions. mVSOP-induced currents were detected even when both intracellular and extracellular alkali ions and divalent cations were replaced by *N*-methyl-D-glutamate (NMDG), or when chloride ions were replaced by methanesulfonate. This result suggested that protons are the permeant ions. To verify this, we measured the reversal potential ($V_{rev}$) by using extracellular and intracellular solutions with different pHs. Tail currents during repolarization to various potentials were elicited after 500 ms of depolarizing prepulses (fig. S2). The $V_{rev}$s shifted in the negative direction as $pH_{in}$ was decreased or $pH_{out}$ was increased (Fig. 2A), and they agreed well with values predicted by the Nernst equation for proton permeability (Fig. 2B). Reversal potentials did not shift from Nernst values even when all NMDG was replaced by the mixture of $K^+$ and $Na^+$ (Fig. 2B), indicating that the channel is selective for protons. To test whether mVSOP-induced currents regulate intracellular pH, changes in intracellular pH during depolarization were measured after acid loading using the pH-sensitive fluorescent dye 2′,7′-bis-(2-carboxyethyl)-5-(6)-carboxyfluorescein–acetoxymethyl ester (BCECF-AM) in HEK293 cells. For acid loading, cells were pretreated with ammonium chloride, then washed with ammonium chloride–free solution (*9*). Some cells expressing mVSOP showed recovery of $pH_{in}$ even without stimulating membrane depolarization by high $K^+$ concentrations. Increasing extracellular potassium concentration after intracellular acidification led to rapid proton efflux in most mVSOP-expressing cells (Fig. 2, C and D). These results suggest that the channels expressed in mVSOP-transfected cells are proton-selective, voltage-dependent channels.

[1]Section of Developmental Neurophysiology, Okazaki Institute for Integrative Bioscience; [2]The Graduate University for Advanced Studies; [3]National Institute for Physiological Sciences, National Institutes of Natural Sciences, Higashiyama 5-1, Myodaiji-cho, Okazaki, Aichi 444-8787, Japan. [4]Neuroscience Research Institute, National Institute of Advanced Industrial Science and Technology, Tsukuba Central 6, Higashi 1-1-1, Tsukuba, Ibaraki 305-8566, Japan.

*To whom correspondence should be addressed. E-mail: yokamura@nips.ac.jp

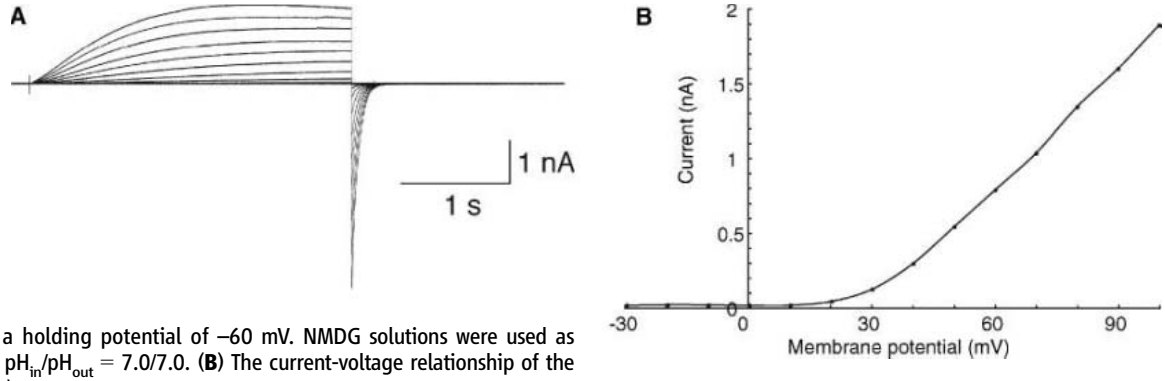


**Fig. 1.** Depolarization-activated whole-cell current induced by mVSOP. (**A**) A family of current traces recorded from an mVSOP-transfected tsA201 cell under voltage clamp in the whole-cell patch configuration. Pulses stepped by 3 s were applied in 10-mV increments ranging from −30 mV to 100 mV from a holding potential of −60 mV. NMDG solutions were used as patch and bath solutions. $pH_{in}/pH_{out}$ = 7.0/7.0. (**B**) The current-voltage relationship of the current traces shown in (A).

Voltage-gated proton channels ($H_v$ channels) have previously been described in mammalian eosinophils (*10*, *11*), macrophages (*12*, *13*), microglia (*14*), and snail neurons (*15*–*17*). A characteristic feature of the native $H_v$ channel is its unique pH dependence of gating (*18*), in which the voltage activation relationship depends strongly on both the intracellular and extracellular pH. Current-voltage

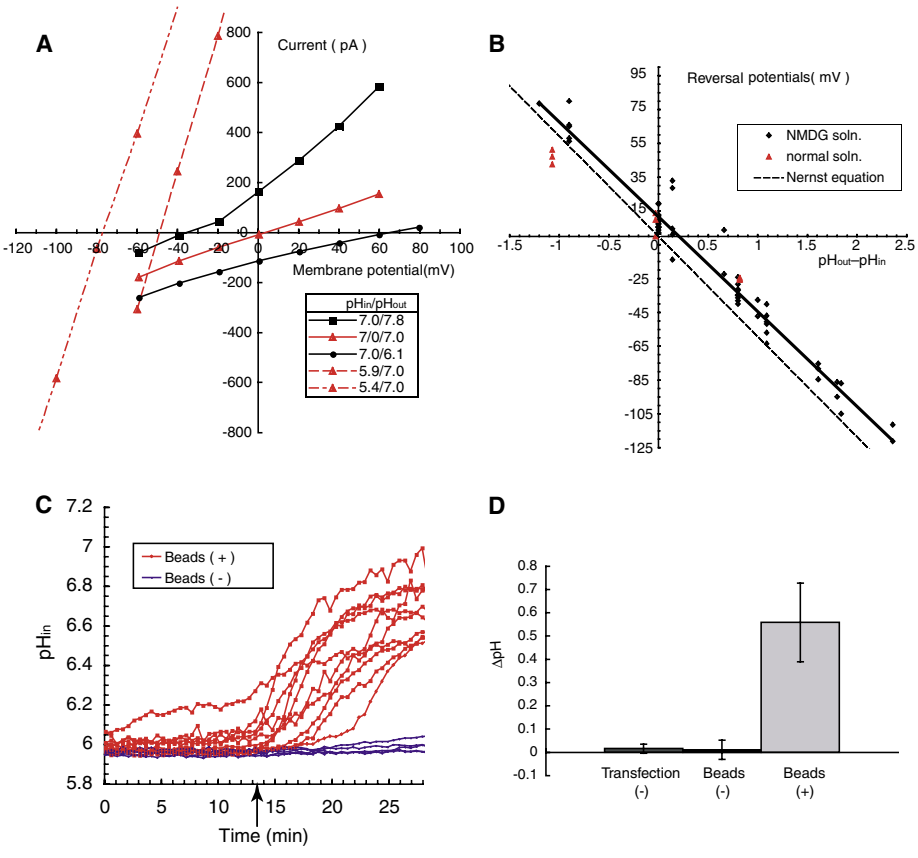


**Fig. 2.** Evidence that VSOP-induced currents are proton selective. (**A**) Plots of tail current amplitude against membrane potential under various pH conditions. Reversal potentials were determined from the intercept of the current-voltage relationship of tail currents. (**B**) Comparison of the reversal potentials obtained from tail currents with proton equilibrium potentials ($E_H$) predicted from Nernst equation. The solid line indicates linear fitting of reversal potentials against ΔpH (56.0 mV/ΔpH). The dashed line shows $E_H$ calculated by the Nernst equation (59.3 mV/ΔpH). Junction potentials ranging up to 4 mV were corrected in the plot. In the normal solution, a mixture of sodium, potassium, and calcium was substituted for NMDG. (**C**) Ratiometric fluorescence measurements with pH-sensitive dye of $pH_{in}$ in mVSOP-transfected cells [beads (+), red] and nontransfected cells [beads (–), blue]. (**D**) Differences of $pH_{in}$ before and after depolarization were quantified. $pH_{in}$ immediately after intracellular acidification by $NH_4Cl$ [time 0 in (C)] and that at 10 min after the start of perfusion [arrow in (C)] of high-potassium solution were measured. Transfection (–) denotes results from cells without transfection ($n$ = 14). Beads (+) denotes results from transfected cells ($n$ = 25). Beads (–) denotes cells that did not express CD8 in the same dish for beads (+) cells ($n$ = 18).

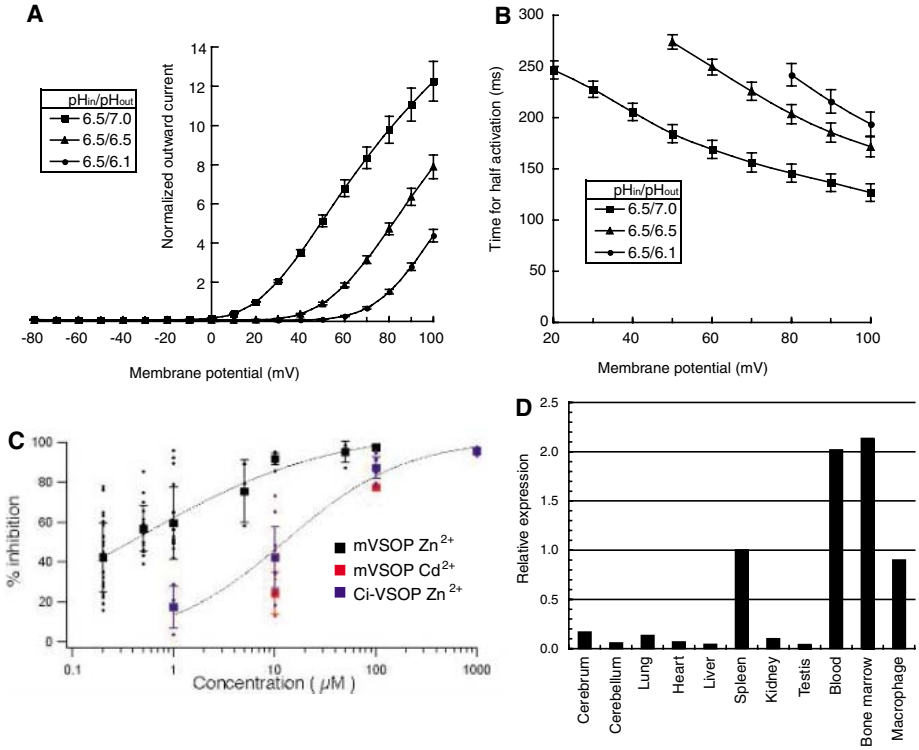


**Fig. 3.** pH-dependent gating and inhibition by divalent metal cations of mVSOP-induced currents. (**A**) The current-voltage relationships evoked by a series of voltage steps in 10-mV increments (–80 to 100 mV) under $pH_{in}$ = 6.5 and $pH_{out}$ = 7.0, 6.5, or 6.1. The pulse duration was 500 ms. Currents were measured from the same sets of cells. Current amplitudes at the end of the depolarizing pulse obtained under each condition of $pH_{out}$ were normalized by those at 20 mV recorded under $pH_{out}$ = 7.0 for individual cells. (**B**) Voltage dependence and pH dependence of the time required for half-maximal activation. Maximal current was measured as the amplitude at the end of depolarizing pulse. The pulse duration was 500 ms. Representative current traces for (A) and (B) are shown in fig. S2. Averaged values ± SE are shown ($n$ = 9) in (A) and (B). (**C**) Dose-response curves of inhibition by zinc and cadmium ions. Small circles are plots from individual cells, and squares denote average values. The holding potential was –80 mV. (**D**) Tissue distributions of VSOP mRNA examined by real-time RT-PCR. The expression level of VSOP mRNA was normalized by expression in the spleen. L8 ribosomal protein was used as internal control.

(*I-V*) relations measured from the same cell at three distinct extracellular pHs showed that when the extracellular pH was decreased from 7.0 to 6.1, the *I-V* curve shifted in the positive direction by about 50 mV (Fig. 3A and fig. S2). When intracellular pH was altered, the *I-V* relationship shifted in the opposite direction by a similar value (fig. S3). Outward currents were activated at a similar threshold of membrane potential ($V_{threshold}$), when measured at two different conditions of extracellular pH = 6.1 and 6.5, with a small pH gradient across the cell membrane (fig. S3). These findings are consistent with the observation that the voltage activation curve is predicted from the gradient between the extracellular and the intracellular pH in native $H_v$ channels (*11*). $V_{threshold}$ was always more positive than $V_{rev}$ within the range of pH gradients we examined ($-1.5 < \Delta pH < 2.5$; $\Delta pH$ is the difference of pH across the cell membrane, $pH_{out} - pH_{in}$). Therefore, mVSOP opens at a range of membrane potentials over the equilibrium potential for protons, thus enabling outwardly rectifying property for proton flow. Activation kinetics were also pH-dependent; the time for half activation at varied membrane potentials became slower as the extracellular pH decreased (Fig. 3B). This pH-dependent gating of VSOP-induced currents agrees well with a reported behavior of native $H_v$ currents (*12*, *18*).

$H_v$ currents are known to be inhibited by submillimolar concentrations of $Zn^{2+}$, $Cd^{2+}$, and other divalent cations (*11*, *19*, *20*). Likewise, mVSOP-derived currents were reversibly decreased by submillimolar concentrations of $Zn^{2+}$ and $Cd^{2+}$ (Fig. 3C and fig. S2). Consistent with results for native $H_v$ currents (*19, 20*), $Zn^{2+}$ had a stronger effect (dissociation constant $K_d = 0.48$ μM) than $Cd^{2+}$.

Previous electrophysiological studies have revealed $H_v$ currents in mammalian blood cells, alveolar epithelium cells of the lung, and microglia of the brain (*11*, *12*, *14*). Consistent with this, quantitative reverse transcriptase polymerase chain reaction (RT-PCR) (Fig. 3D) showed marked gene expression of mVSOP in the spleen, whole blood, bone marrow, and macrophages.

Does mVSOP directly encode $H_v$ channels, or just activate endogenous $H_v$ channels? To address this, we tested whether modification of molecular structure leads to changes in the properties of the $H_v$ current. Changes in voltage dependence were examined for mutations at each of the three positively charged amino acids of the S4-like segment. The conductance-voltage (*G-V*) curve was obtained with the same pH (7.0) in both the extracellular and intracellular solutions. In the R204Q mutant, where $Arg^{204}$ is replaced by Gln, detailed analysis of current kinetics was not possible due to a low level of protein expression to the cell surface. The *G-V* curve for R207Q was indistinguishable from that of the wild-type channel (fig. S4), whereas R201Q showed much faster kinetics of activation (Fig. 4A). The *G-V* curve of the R201Q mutant was shifted in a negative direction by about 50 mV (Fig. 4B). The steepness of activation (*z* value) became slightly sharper (1.9 ± 0.25 for R201Q, $n = 9$ cells, versus 1.4 ± 0.15 for wild type, $n = 5$). In this mutant, $V_{theshold}$ was more negative than $V_{rev}$, suggesting that inward current flows at a membrane potential more negative than $V_{rev}$. In fact, an inward proton current was clearly seen at acidic extracellular pH (Fig. 4, C and D). R201 may therefore help the channel to maintain a $V_{threshold}$ that is more positive than the equilibrium potential for protons, which enables the outwardly-rectifying property of the channel.

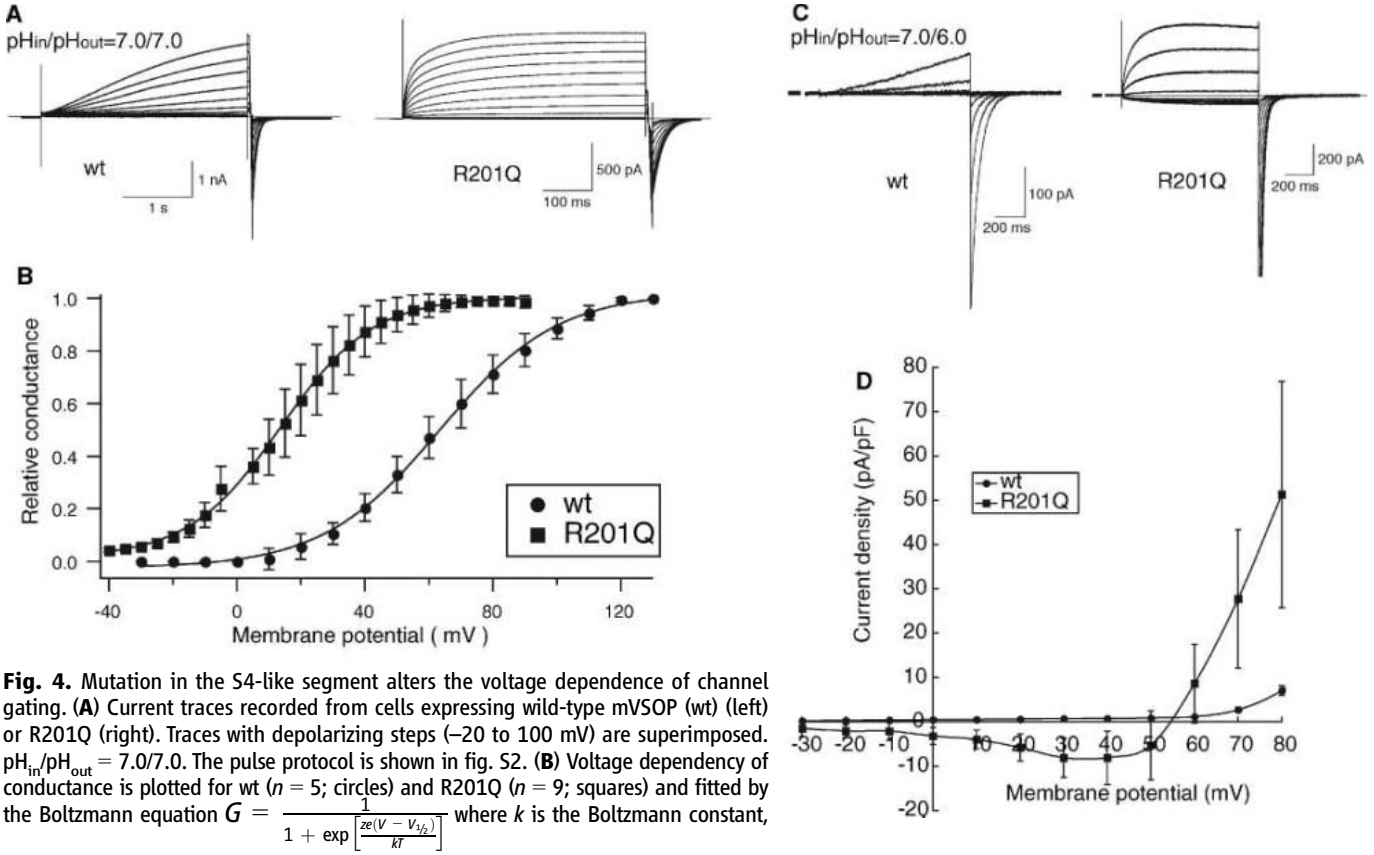


**Fig. 4.** Mutation in the S4-like segment alters the voltage dependence of channel gating. (**A**) Current traces recorded from cells expressing wild-type mVSOP (wt) (left) or R201Q (right). Traces with depolarizing steps (–20 to 100 mV) are superimposed. $pH_{in}/pH_{out} = 7.0/7.0$. The pulse protocol is shown in fig. S2. (**B**) Voltage dependency of conductance is plotted for wt ($n = 5$; circles) and R201Q ($n = 9$; squares) and fitted by the Boltzmann equation $G = \frac{1}{1 + \exp\left[\frac{ze(V - V_{1/2})}{kT}\right]}$ where *k* is the Boltzmann constant, *e* is the elementary electric charge, *T* is temperature, and *z* is the valence. $V_{1/2}$ values are 63.7 ± 7.6 mV and 14.8 ± 8.0 mV, and *z* values are 1.4 ± 0.15 and 1.9 ± 0.25 for wt and R201Q, respectively. Error bars indicate SD. (**C**) Current traces under $pH_{in}/pH_{out} = 7.0/6.1$ for wt (left) and R201Q (right). Traces with depolarizing steps (20 to 90 mV) are superimposed. In R201Q, tail currents are scaled out in this magnification. (**D**) The current-voltage relationships for wt ($n = 8$) and R201Q mutant ($n = 12$) in $pH_{in}/pH_{out} = 7.0/6.1$. Averaged current densities at the end of depolarization pulses are plotted. Inward current is evident for R201Q. In [(A) to (D)], the holding potential was –60 mV. Error bars indicate SD.

The ortholog of mVSOP was also isolated from an ascidian, *C. intestinalis* (called Ci-VSOP). Ci-VSOP showed marked homology to mVSOP in the putative S1 to S4 segments, although the N-terminal and C-terminal cytoplasmic regions of the two VSOP proteins were highly divergent (fig. S1). This ascidian ortholog protein also exhibited outward-rectifying proton currents activated by membrane depolarization when expressed in tsA201 cells. Ci-VSOP showed 27 times lower sensitivity to $Zn^{2+}$ (Fig. 3C) and significantly faster activation kinetics than mVSOP (fig. S5). The shifted voltage dependency of activation in the mutant R201Q of mVSOP, together with the differences in kinetics and pharmacology between ascidian and mammalian orthologs, suggest that VSOP proteins are the principal subunit of the $H_v$ channel, rather than a regulator or accessory subunit of an endogenous proton channel.

We identified a protein consisting primarily of a VSD as an $H_v$ channel, providing the first example of a protein in which the VSD functions beyond voltage sensing. Proton efflux through $H_v$ channels, accompanied by membrane depolarization, facilitates acute production of reactive oxygen species in phagocytosis (*11*, *14*, *21*). Further studies of VSOP will not only provide new clues to general principles of proton permeation and gating of membrane proteins (*10*, *11*, *14*), but may also open avenues for advances in understanding biological events related to respiratory burst and phagocytosis.

*Note added in proof*: D. Clapham's laboratory reports similar properties of a human ortholog, $H_v1$ (*22*).

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**Supporting Online Material**

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Materials and Methods
Figs. S1 to S5
References


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# SV2 Is the Protein Receptor for Botulinum Neurotoxin A


**Min Dong,[1] Felix Yeh,[1,2] William H. Tepp,[3] Camin Dean,[1] Eric A. Johnson,[3] Roger Janz,[4] Edwin R. Chapman[1,2]***



**How the widely used botulinum neurotoxin A (BoNT/A) recognizes and enters neurons is poorly understood. We found that BoNT/A enters neurons by binding to the synaptic vesicle protein SV2 (isoforms A, B, and C). Fragments of SV2 that harbor the toxin interaction domain inhibited BoNT/A from binding to neurons. BoNT/A binding to SV2A and SV2B knockout hippocampal neurons was abolished and was restored by expressing SV2A, SV2B, or SV2C. Reduction of SV2 expression in PC12 and Neuro-2a cells also inhibited entry of BoNT/A, which could be restored by expressing SV2 isoforms. Finally, mice that lacked an SV2 isoform (SV2B) displayed reduced sensitivity to BoNT/A. Thus, SV2 acts as the protein receptor for BoNT/A.**


Botulinum neurotoxin A (BoNT/A) is one of seven neurotoxins (designated BoNT/A to BoNT/G) produced by the bacterium *Clostridium botulinum* (*1*). BoNT/A blocks neurotransmitter release by cleaving synaptosome-associated protein of 25 kD (SNAP-25) (*2*, *3*) within presynaptic nerve terminals. Owing to its long-lasting effects, BoNT/A is used to treat a wide range of medical conditions and has also emerged as a potential biological weapon (*4*, *5*).

It has been proposed that receptors for BoNTs are composed of both gangliosides that bind toxins with low affinity and protein receptors that form high-affinity complexes with toxins (*6*, *7*). Synaptotagmins I and II, two homologous synaptic vesicle proteins, have been shown to function in conjunction with gangliosides to mediate entry of BoNT/B into cells (*8*–*11*). Low-affinity interactions between BoNT/A and gangliosides have also been reported (*12*–*15*), and depletion of gangliosides in neuroblastoma cells prevented entry of BoNT/A (*16*). In addition, mice lacking complex gangliosides display reduced sensitivity to BoNT/A (*10*, *17*). Protein components are certainly required for BoNT/A entry into nerve terminals but have not been identified (*1*, *6*, *7*).

To identify protein receptors for BoNT/A, we studied the toxin's route of entry. Stimulation of neurotransmitter release is known to reduce the time required for paralysis caused by BoNT/A at the diaphragm (*18*). We found that depolarization with a high-$K^+$ solution in hemidiaphragm preparations increased toxin binding to neuromuscular junctions (NMJs) by a factor of ~6 (Fig. 1A), suggesting that


[1]Howard Hughes Medical Institute and Department of Physiology, [2]Molecular and Cellular Pharmacology Program, [3]Department of Food Microbiology and Toxicology, University of Wisconsin, Madison, WI 53706, USA. [4]W. M. Keck Center for Learning and Memory and Department of Neurobiology and Anatomy, University of Texas–Houston Medical School, Houston, TX 77030, USA.

*To whom correspondence should be addressed: E-mail: chapman@physiology.wisc.edu

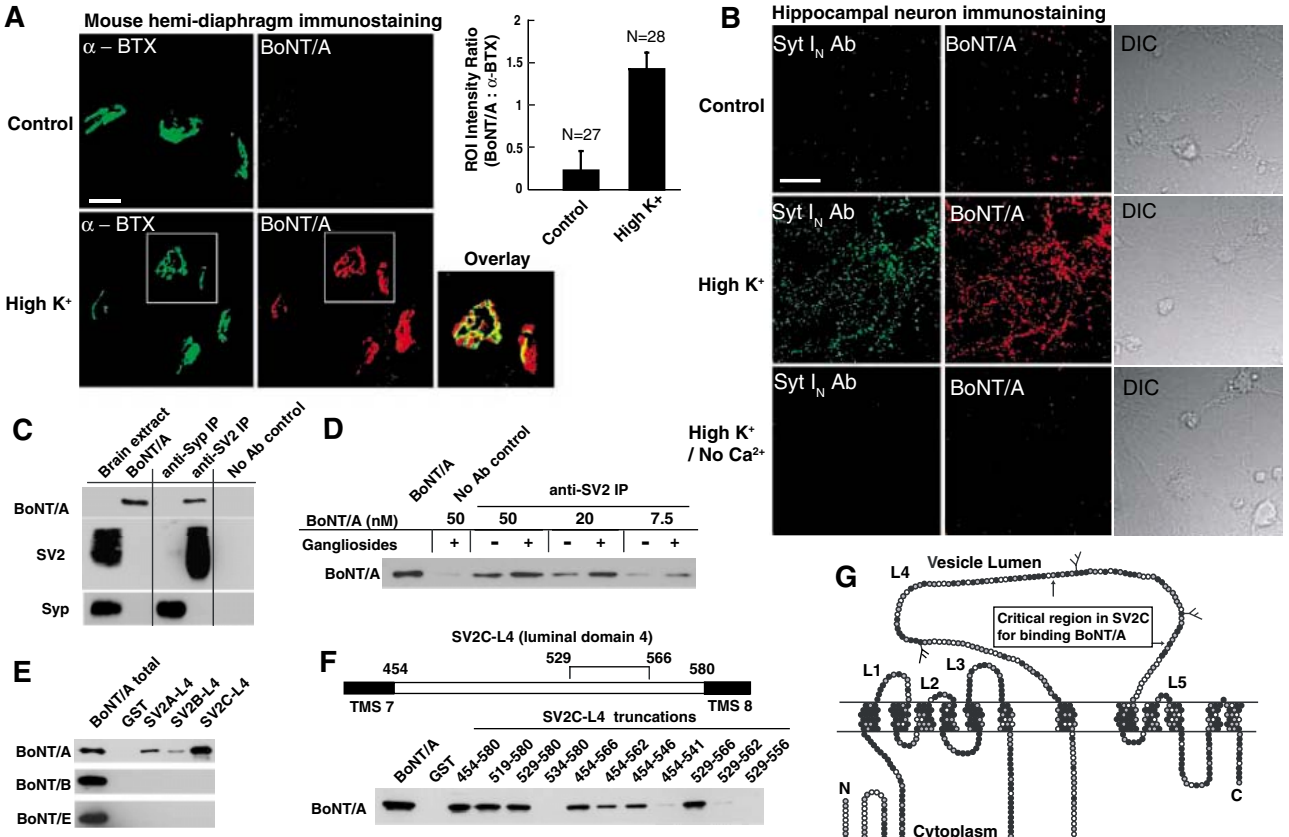


**Fig. 1.** BoNT/A enters neurons by way of recycling secretory vesicles and interacts with a luminal domain of the synaptic vesicle membrane protein SV2. (**A**) Stimulation of mouse hemidiaphragm preparations with high-$K^+$ buffer increases binding of BoNT/A. NMJs were labeled with α-bungarotoxin (α-BTX). The overlay shows an enlarged image of the region indicated by rectangles. Error bars show SEM ($P < 0.0001$, Student's $t$ test, $n = 27$ images). Scale bar, 20 μm. ROI, region of interest. (**B**) Cultured rat hippocampal neurons were exposed to BoNT/A plus Syt $I_N$ Ab for 1 min in three indicated buffer conditions. Scale bar, 20 μm. DIC, differential interference contrast. (**C**) Immunoprecipitation (IP) of Syp and SV2 from rat brain detergent extracts that contained BoNT/A. (**D**) Coimmunoprecipitation of BoNT/A and SV2 from mouse brain detergent extracts was carried out with or without exogenous gangliosides. (**E**) The fourth luminal domain (SV2-L4) of all three SV2 isoforms was fused to glutathione *S*-transferase (GST), immobilized, and used in binding assays with soluble BoNT/A. (**F**) The critical region for BoNT/A binding was mapped to a short fragment in the SV2C luminal domain with the use of GST fusion protein pull-down assays. TMS, transmembrane segment. (**G**) Model of SV2 topology. Black circles indicate conserved residues in all SV2 isoforms, gray circles are residues conserved in two SV2 isoforms, and open circles represent nonconserved residues.

synaptic vesicle exocytosis exposes receptors for BoNT/A.

Using cultured rat hippocampal neurons as a model system, we found that BoNT/A was taken up in an activity-dependent manner along with an antibody that recognizes the luminal domain of secretory vesicle protein synaptotagmin I (Syt $I_N$ Ab) (*11*, *19*) (Fig. 1B); BoNT/A and the antibody were largely colocalized (fig. S1A). In addition, depolarization resulted in increased cleavage of SNAP-25 by BoNT/A in hippocampal neurons (fig. S1B) and in cultured spinal cord neurons (*20*). Finally, pretreating neurons with BoNT/B, which blocks synaptic vesicle exocytosis by cleaving Synaptobrevin (Syb) (*21*), abolished uptake of BoNT/A (fig. S1C). Thus, the BoNT/A receptor is localized to Syb-containing secretory vesicles, which include synaptic vesicles and large dense core vesicles. All subsequent experiments, which used hippocampal neurons and hemidiaphragms, were carried out with the use of high $K^+$ buffer to drive BoNT/A receptors to the cell surface.

We immunoprecipitated known synaptic vesicle proteins from rat brain detergent extracts and assayed the precipitates for bound toxin (*22*). In this screen, an antibody that recognizes all isoforms of SV2 [pan-SV2 (*23*, *24*)] was able to coimmunoprecipitate BoNT/A (Fig. 1C). An antibody against another abundant integral synaptic vesicle membrane protein—synaptophysin (Syp)—failed to coimmunoprecipitate BoNT/A (Fig. 1C). The addition of exogenous gangliosides increased the level of coimmunoprecipitation of BoNT/A with SV2 (Fig. 1D), particularly at lower toxin concentrations, indicating that gangliosides can promote the formation of stable BoNT/A•SV2 complexes.

SV2 is a conserved integral membrane protein expressed in vertebrates and is localized to synaptic and endocrine secretory vesicles (*22*). Three highly homologous isoforms have been identified: SV2A, SV2B, and SV2C (*22*). SV2A and SV2B are widely expressed in the brain, whereas SV2C is more restricted to evolutionarily older brain regions (*24*, *25*). SV2 is a glycoprotein with 12 putative transmembrane domains (Fig. 1G). BoNT/A bound directly to the largest luminal loop (L4) of SV2A, SV2B, and SV2C; BoNT/B and BoNT/E failed to bind (Fig. 1E). The luminal domains of other major synaptic vesicle membrane proteins—including synaptogyrin 1, synaptogyrin 3, SVOP, and Syp—did not bind BoNT/A (fig. S2A). SV2C showed the most robust BoNT/A binding activity (Fig. 1E). A short fragment (amino acids 529 to 566) within the SV2C-L4 loop was able to pull down levels of BoNT/A similar to the full-length L4 loop (Fig. 1, F and G). A sequence alignment between SV2A, SV2B, and SV2C, within this region, is shown in fig. S2B.

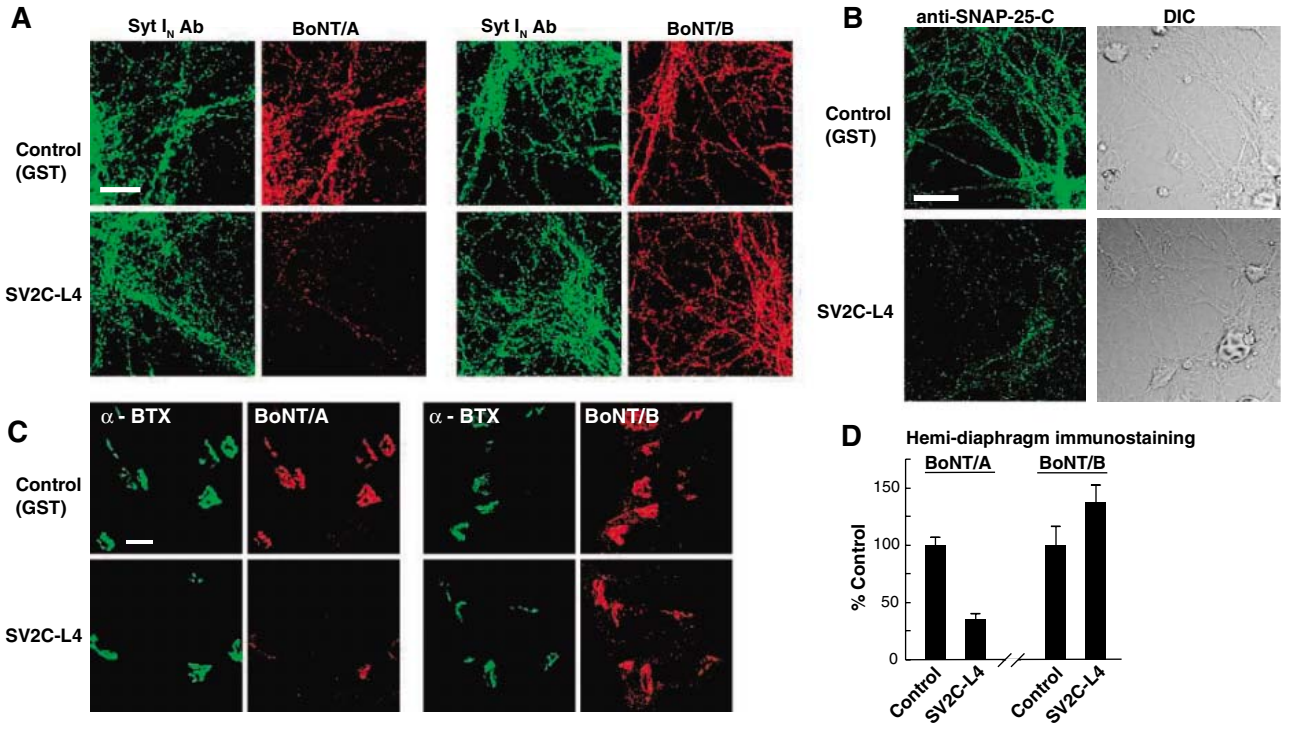


**Fig. 2.** SV2C-L4 inhibits binding and entry of BoNT/A into hippocampal neurons and motor nerve terminals. (**A**) Hippocampal neurons and (**C**) mouse hemidiaphragm preparations were exposed to BoNT/A (or BoNT/B) and Syt $I_N$ Ab in the presence of either GST alone or SV2C-L4. (**B**) Cleavage of SNAP-25 by BoNT/A was detected with the use of an antibody (anti–SNAP-25-C) that recognizes only the cleaved form of SNAP-25. (**D**) SV2C-L4 reduced BoNT/A binding to mouse hemidiaphragm preparations by 65% ($P < 0.0001$, $t$ test, $n = 76$), but did not affect binding of BoNT/B ($P > 0.05$, $t$ test, $n = 49$). Error bars show SEM.

Recombinant SV2C-L4 fragments reduced BoNT/A binding to cultured hippocampal neurons but did not affect Syt $I_N$ Ab, BoNT/B, or BoNT/E uptake (Fig. 2A and fig. S3C). Preincubation of BoNT/A with SV2C-L4 also reduced BoNT/A binding to NMJs, with no effect on the binding of BoNT/B (Fig. 2, C and D). BoNT/B is an appropriate control because it also enters by binding to a synaptic vesicle protein (*8*, *11*). Binding of BoNT/B was inhibited by adding a peptide (P21) derived from its receptor, synaptotagmin II (*11*), but this peptide did not affect BoNT/A binding to hippocampal neurons (fig. S2, A and B). Furthermore, using an antibody that only recognizes the cleaved form of SNAP-25, we found that SV2C-L4 inhibited the functional entry of BoNT/A into these neurons, as evidenced by reduced cleavage of SNAP-25 (Fig. 2B).

SV2A and SV2B single-knockout mice and SV2A and SV2B double-knockout mice have been generated (*26*, *27*). Although mice lacking SV2A display severe seizures and die within 2 to 3 weeks of birth (*26*), cultured hippocampal neurons from SV2A and SV2B double-knockout mice develop normal synaptic structures and are capable of releasing neurotransmitters (*26*, *28*). Because hippocampal neurons express SV2A and SV2B, but not SV2C (*24*, *25*) (fig. S4, A and B), neurons from SV2A and SV2B double-knockout mice serve as an ideal loss-of-function model to study the potential role of SV2 as the receptor for BoNT/A.

We tested the function of SV2B by comparing BoNT/A binding with neurons from SV2B knockout mice ($SV2B^{-/-}$) and their wild-type littermates. Neurons were simultaneously exposed to BoNT/A and BoNT/B. SV2B knockout neurons showed reduced binding of BoNT/A as compared with that of wild-type neurons (28% reduction) (Fig. 3A). We next asked whether the remaining BoNT/A binding activity was mediated by SV2A. $SV2A^{+/-}SV2B^{-/-}$ mice were bred with each other, and therefore all of the progeny were $SV2B^{-/-}$, but they had varying levels of SV2A: $SV2A^{+/+}$, $SV2A^{+/-}$, and $SV2A^{-/-}$ (Fig. 3B). Binding of BoNT/A to $SV2A^{+/-}SV2B^{-/-}$ neurons was reduced by 53% as compared with that of $SV2A^{+/+}SV2B^{-/-}$ neurons (Fig. 3C). BoNT/A did not bind to SV2A and SV2B double-knockout neurons (Fig. 3, B and C). Binding of BoNT/B to neurons of each genotype remained the same (Fig. 3, A to C).

We carried out rescue experiments by transfecting SV2A, SV2B, or SV2C into SV2A and SV2B double-knockout neurons, using a vector that expresses both SV2 and green fluorescent protein (GFP) under the control of separate promoters. Binding of BoNT/A was observed only in neurons that expressed SV2A, SV2B, or SV2C (Fig. 3D and fig. S6A). Enlarged, overlaid images showed a high degree of colocalization between SV2 and BoNT/A at synapses of transfected neurons (Fig. 3D and fig. S6A, overlay).

In addition to neurons, BoNT/A can enter cell lines, including PC12 (*11*) and Neuro-2a cells (*16*). These cell lines provide homogeneous model systems to study SV2 function in which the expression of SV2 isoforms can be readily altered. PC12 cells express only SV2A (*25*) (fig. S4A) and Neuro-2a cells express only SV2C (fig. S4A). We established three stable SV2A knockdown PC12 cell lines (Fig. 3E). All three knockdown cell lines were relatively resistant to BoNT/A entry, as evidenced by reduced cleavage of SNAP-25 (Fig. 3E). Transient transfection of SV2B or SV2C in an SV2A knockdown cell line (number 16) rescued BoNT/A entry (Fig. 3E). Similarly, knockdown of SV2C in Neuro-2a cells inhibited entry of BoNT/A (Fig. 3F). This defect was rescued by expressing rat SV2A or SV2B GFP fusion proteins in these cells (fig. S6B). An enlarged confocal section of transfected cells revealed a high degree of colocalization between SV2A-GFP and internalized BoNT/A (fig. S6C).

Disruption of neurotransmitter release at diaphragm motor nerve terminals is the cause of death from BoNT poisoning (*29*). All three SV2 isoforms were detected by immunostaining at diaphragm motor nerve terminals (fig. S4C). Because SV2C knockout mice are currently not available and SV2A and SV2B

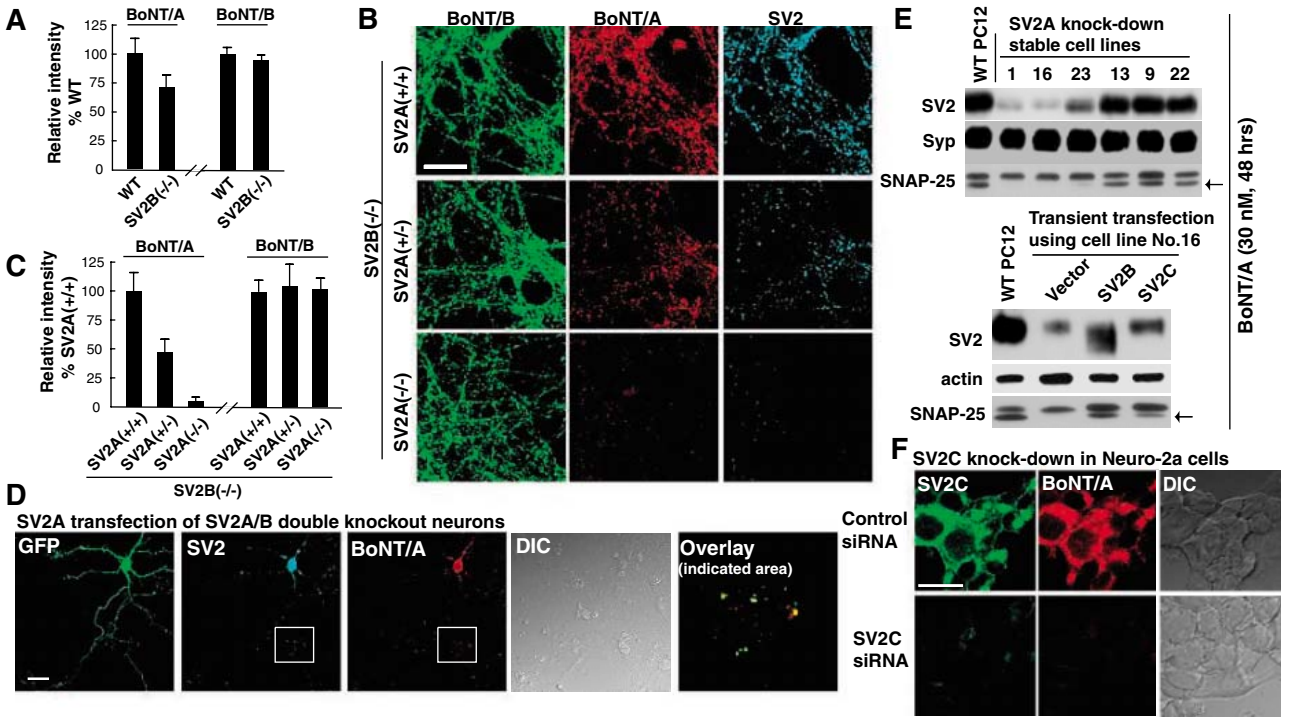


**Fig. 3.** SV2 expression is required for BoNT/A binding and entry into cells. (**A**) Hippocampal neurons from SV2B knockout ($SV2B^{-/-}$) mice displayed reduced binding of BoNT/A (28% reduction, $P < 0.0001$, $t$ test, $n = 18$) compared with neurons from wild-type (WT) littermates. Binding of BoNT/B remained the same ($P > 0.05$, $t$ test, $n = 22$). Error bars show SD. (**B**) Hippocampal neurons from littermates with the indicated genotypes were exposed to BoNT/A and BoNT/B. Triple immunostaining was performed. (**C**) $SV2A^{+/-}SV2B^{-/-}$ neurons displayed a 53% reduction of BoNT/A binding activity compared with $SV2A^{+/+}SV2B^{-/-}$ ($P < 0.0001$, $t$ test, $n = 11$); BoNT/A did not bind to $SV2A^{-/-}SV2B^{-/-}$ double-knockout neurons. The binding of BoNT/B remained the same for all genotypes ($P > 0.05$, $t$ test, $n = 11$). Error bars show SD. (**D**) Rat SV2A was expressed in $SV2A^{-/-}SV2B^{-/-}$ double-knockout hippocampal neurons. BoNT/A selectively bound to cells that expressed SV2A. The overlay shows an enlarged image of the region indicated by a rectangle. (The color of the SV2 signal was changed from cyan to green to visualize colocalization with red BoNT/A signals.) (**E**) (Top) A short hairpin–mediated RNA (shRNA) construct was transfected into PC12 cells to establish stable SV2A knockdown cell lines. The indicated cell lines were exposed to BoNT/A, and cell lysates were analyzed by Western blot; the arrow indicates the cleaved form of SNAP-25. Syp served as a loading control. (Bottom) An SV2A knockdown PC12 cell line (number 16) was transiently transfected with rat SV2B or SV2C. Cleavage of SNAP-25 by BoNT/A was assayed as described for the top panel. Actin served as a loading control. (**F**) Neuro-2a cells were transfected with either a pool of synthesized SV2C small interfering RNA (siRNA) that target the mouse SV2C coding sequence or with a pool of control siRNA that had random sequences. SV2C siRNA inhibited SV2C expression in transfected cells, and these cells failed to take up BoNT/A.

double-knockout mice have severe seizures, we studied binding of BoNT/A to diaphragm preparations from $SV2A^{+/-}$ $SV2B^{-/-}$ mice (*26*). These mice are healthy and have the least amount of SV2 expression available (fig. S4, D and E). Binding of BoNT/A to NMJs of these mice was reduced by 72% as compared with that of wild-type controls (Fig. 4, A and B).

Finally, we carried out whole-animal studies to determine whether changes in SV2 expression in mice altered their susceptibility to BoNT/A. To minimize potential defects in synaptic transmission in vivo, we compared the BoNT/A sensitivity of SV2B knockout mice to their wild-type littermates, because SV2B knockout mice are phenotypically normal (*26*). Sensitivity to BoNT/A was assessed with an established rapid assay, in which large doses of toxin are injected intravenously (*10*, *11*, *30*). The survival time of each mouse was determined and converted to intraperitoneal toxicity (*30*). SV2B knockout mice survived significantly longer

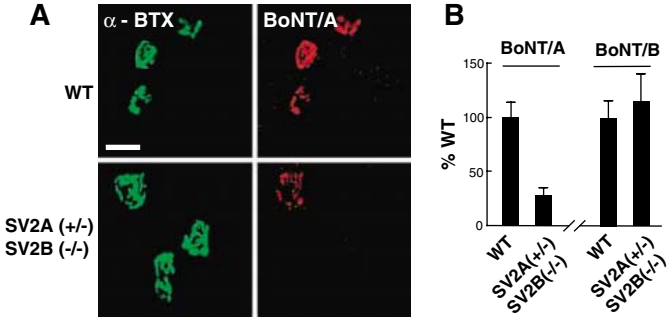


**Fig. 4.** SV2 knockout mice have reduced BoNT/A binding activity at diaphragm motor nerve terminals and are less sensitive to BoNT/A. (**A** and **B**) Binding of BoNT/A to mouse hemidiaphragms, prepared from wild-type (WT) and $SV2A^{+/-}SV2B^{-/-}$ mice, was assayed as described in Fig. 1A. $SV2A^{+/-}SV2B^{-/-}$ mice exhibit reduced BoNT/A binding activity compared with that of wild-type mice (72% reduction, $P < 0.001$, $t$ test, $n = 47$), whereas binding of BoNT/B was the same ($P > 0.05$, $t$ test, $n = 20$). Error bars show SEM. (**C**) The same amount of BoNT/A was injected into $SV2B^{-/-}$ mice and their wild-type littermates. $SV2B^{-/-}$ mice live longer than wild-type mice (43 min versus 33 min, $P < 0.05$, paired $t$ test), corresponding to a 60% loss in toxicity in the SV2B knockout mice (*30*). $LD_{50}$, mean lethal dose.

**C** Rapid toxicity assay

| Genotype (littermates) | Time-to-death (min) Pair 1 | 2 | 3 | 4 | Average effective toxicity ($LD_{50}$/ml) |
|---|---|---|---|---|---|
| WT | 27 | 36 | 35 | 33 | $8.4 \pm 2.9 \times 10^5$ |
| SV2B (-/-) | 44 | 46 | 42 | 41 | $3.4 \pm 0.5 \times 10^5$ |

than wild-type littermates in this assay. The effective toxin concentration in SV2B knockout mice was reduced by 60% as compared with that of wild-type mice (Fig. 4C). The remaining toxicity in SV2B$^{-/-}$ mice was probably mediated by SV2A and SV2C, which are not altered, and by gangliosides, which serve as low-affinity receptors.

By using the secretory vesicle protein SV2 as its protein receptor, BoNT/A attacks active neurons with high selectivity because active neurons expose more receptors during exocytosis. Because BoNT/A blocks vesicle exocytosis, theoretically, a successful BoNT/A entry event will shut down the subsequent exposure of more receptors, allowing toxin molecules to enter active synapses that have yet to be poisoned. It has been shown that BoNT/B uses synaptotagmins I and II to enter cells (*8*, *9*, *11*). BoNT/G has also been reported to bind synaptotagmins I and II (*31*). Thus, secretory vesicle recycling pathways may have been exploited by several BoNTs, thereby contributing to the efficiency of this class of toxins.

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**Supporting Online Material**

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Materials and Methods
Figs. S1 to S6
References


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# Retinoid Signaling Determines Germ Cell Fate in Mice


**Josephine Bowles,[1] Deon Knight,[1] Christopher Smith,[1]* Dagmar Wilhelm,[1] Joy Richman,[1,3] Satoru Mamiya,[4] Kenta Yashiro,[4] Kallayanee Chawengsaksophak,[5] Megan J. Wilson,[1]† Janet Rossant,[5] Hiroshi Hamada,[4] Peter Koopman[1,2]‡**



**Germ cells in the mouse embryo can develop as oocytes or spermatogonia, depending on molecular cues that have not been identified. We found that retinoic acid, produced by mesonephroi of both sexes, causes germ cells in the ovary to enter meiosis and inititate oogenesis. Meiosis is retarded in the fetal testis by the action of the retinoid-degrading enzyme CYP26B1, ultimately leading to spermatogenesis. In testes of *Cyp26b1*-knockout mouse embryos, germ cells enter meiosis precociously, as if in a normal ovary. Thus, precise regulation of retinoid levels during fetal gonad development provides the molecular control mechanism that specifies germ cell fate.**


The ability to generate haploid gametes by meiosis is a unique property of germ cells and is critical for sexual reproduction. Whether germ cells develop as oocytes or spermatogonia depends on the time at which they enter meiosis: If meiosis begins during fetal development, as occurs in the mouse ovary around 13.5 days postcoitum (dpc), oogenesis is triggered, whereas germ cells that delay the onset of meiosis until after birth, as occurs in the testis, adopt a spermatogenic fate (*1*). It is widely believed that fetal germ cells are intrinsically programmed to enter meiosis and initiate oogenesis unless specifically prevented from doing so by a putative "meiosis-inhibiting factor" (*2*). However, such a substance has not been identified in vivo, and the molecular mechanisms regulating entry into meiosis in the fetal ovary but not in the fetal testis are unclear.

We conducted an expression screen designed to identify genes expressed sex-specifically during mouse gonadogenesis (*3*). One of these, *Cyp26b1*, was initially expressed in gonads of both sexes but became strikingly male-specific by 12.5 dpc (Fig. 1, A to C). Section in situ hybridization revealed that *Cyp26b1* is expressed in nascent testis cords (Fig. 1D). Because these contain only two cell types, Sertoli and germ cells, and expression was maintained in $W^e/W^e$ mutant gonads that lack germ cells (*4*) (Fig. 1E), it is clear that Sertoli cells are responsible for *Cyp26b1* expression. *Cyp26b1* was also expressed in some unidentified interstitial cells (Fig. 1D). By using quantitative reverse transcription polymerase chain reaction (RT-PCR), we found maximal expression of *Cyp26b1* at 13.5 dpc in male gonads (fig. S1).

*Cyp26b1* encodes a P450 cytochrome enzyme that degrades the potent morphogen retinoic acid (RA) (*5*). RA regulates the de-


[1]Division of Genetics and Developmental Biology, [2]Australian Research Council Centre of Excellence in Biotechnology and Development, Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD 4072, Australia. [3]Department of Oral Health Science, Faculty of Dentistry, University of British Columbia, Vancouver, BC V6T 1Z3, Canada. [4]Division of Molecular Biology, Institute for Molecular and Cellular Biology, Osaka University, and Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Corporation (JST), Osaka 565-0871, Japan. [5]Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada.

*Present address: Faculty of Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada.
†Present address: Biochemistry Department, University of Otago, Post Office Box 56, Dunedin, New Zealand.
‡To whom correspondence should be addressed. E-mail: p.koopman@imb.uq.edu.au

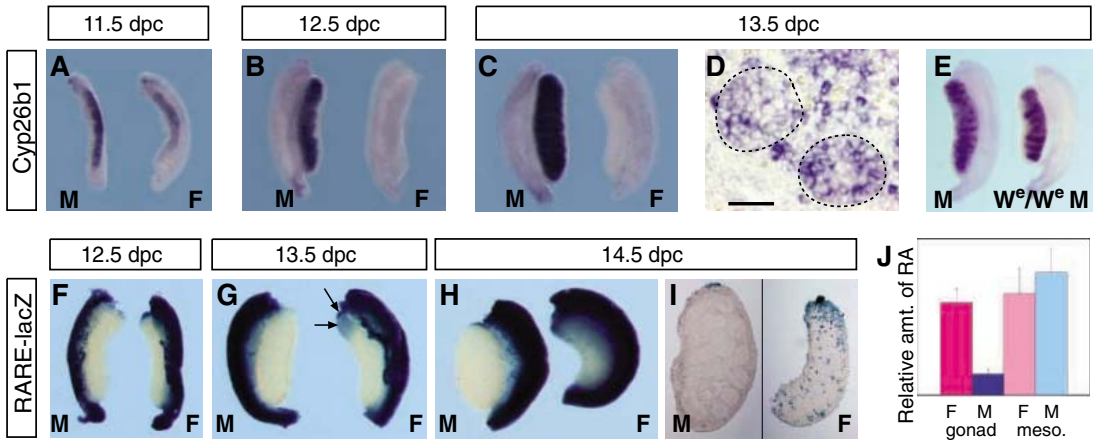


**Fig. 1.** CYP26B1 selectively removes RA from the developing testis. M, male; F, female. *Cyp26b1* expression in mouse UGRs at (**A**) 11.5 dpc, (**B**) 12.5 dpc, and (**C**) 13.5 dpc; (**D**) in a testis section at 13.5 dpc (two cords are outlined; scale bar indicates 50 μm); and (**E**) in $W^e/W^e$ male UGRs at 13.5 dpc. *LacZ* reporter gene expression in RARE-LacZ transgenic mouse UGRs at (**F**) 12.5 dpc, (**G**) 13.5 dpc, and (**H**) 14.5 dpc and (**I**) in gonads separated from mesonephroi at 14.5 dpc (10-μm section). RA response is seen in the fetal ovary at 12.5, 13.5 [anterior, arrows in (G)], and 14.5 dpc (scattered throughout). (**J**) Quantitation of RA in extracts of 13.5-dpc gonads and mesonephroi using F9 RA indicator cells (arbitrary units). Error bars represent 1 SD ($n$ = 3).

velopment of many organ systems, with local concentrations controlled by a balance of synthesis and degradation (*6*–*8*). The strong, male-specific expression of *Cyp26b1* in developing gonads is consistent with recent data implicating RA in the timing of meiotic initiation (*9*) and prompted us to investigate whether CYP26B1 might be the meiosis-inhibiting factor in male embryos.

To test for the presence of RA in the developing urogenital system, we used a mouse strain in which *lacZ* reporter gene expression is controlled by a RA response element (RARE-LacZ mice) (*10*). Strong *lacZ* staining was detected in mesonephroi of both sexes (Fig. 1, F to H). Little or no staining was found in developing testes (Fig. 1, F to I). In the ovary, *lacZ* staining, indicating a transcriptional response to endogenous RA, was seen in cells at the anterior pole at 12.5 (Fig. 1F), 13.5 (Fig. 1G), and 14.5 dpc (Fig. 1H) and also in scattered cells throughout the gonad at 14.5 dpc (Fig. 1, H and I). The relatively weak lacZ staining in these ovaries may reflect a technical limitation or an artifact of the transgenic indicator mouse line used (*10*), because quantitative measurements in gonad extracts revealed high levels of RA in the ovary (Fig. 1J). Extracts from male and female mesonephroi also contained high levels of RA, whereas testis extracts elicited a weak response (Fig. 1J).

To identify the source of the RA, we cultured explanted tissues on a lawn of RA-sensitive reporter cells overnight, removed them, and stained the reporter cells for LacZ expression; under these conditions, only sustained synthesis of RA (as opposed to residual RA content) elicits a response. Mesonephroi from 11.5 (Fig. 2A) and 12.5 (*11*) dpc embryos stimulated abundant *lacZ* gene activity in this assay, but male or female gonads at those stages did not, identifying the mesonephroi as sites of RA synthesis and hence the source of RA (Fig. 2B) in the developing urogenital system. Accordingly, we also found robust expression of *Aldh1a2*, encoding the major enzyme of RA synthesis in the mouse embryo (ALDH1A2, also called RALDH2) (*12*), in mesonephroi, but not gonads, of both sexes as early as 10.5 dpc, and persisting until at least 13.5 dpc (Fig. 2, C and D). These observations suggest a source-sink regulatory system (Fig. 2, E and F) controls sex-specific RA levels in fetal gonads.

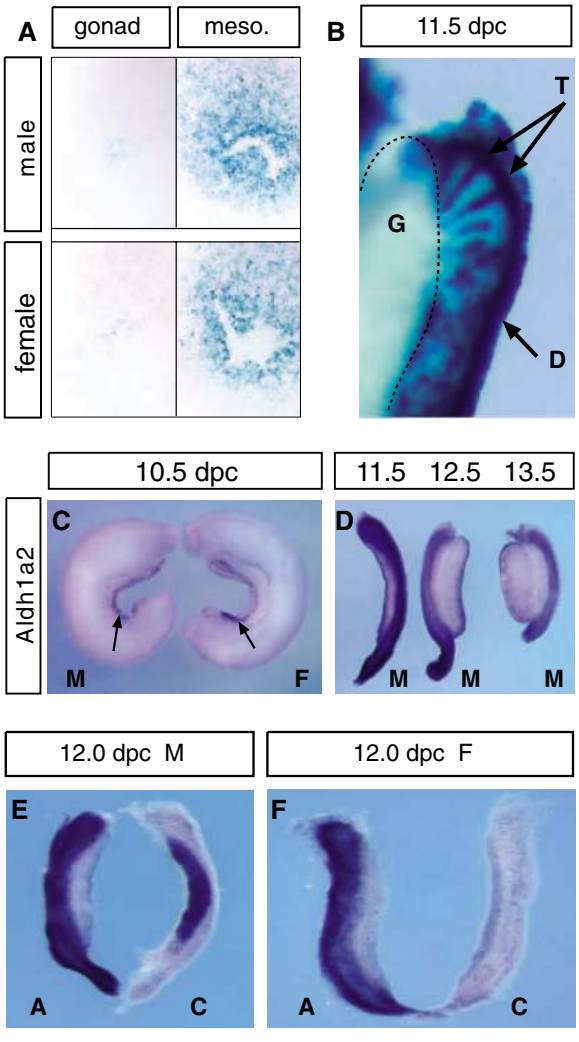


**Fig. 2.** The mesonephros is the source of RA in the developing urogenital system. (**A**) Detection of RA secreted over a 24-hour period by 11.5-dpc gonads and mesonephroi using F9 RA indicator cells. (**B**) *LacZ* reporter expression in mesonephric duct (D) and tubules (T) of an 11.5-dpc female RARE-LacZ UGR. The gonad (G) is outlined. (**C**) Expression of *Aldh1a2* in 10.5-dpc UGRs (attached to trunk of embryo; arrows indicate mesonephroi) and (**D**) in 11.5-, 12.5-, and 13.5-dpc male UGRs. Expression of *Aldh1a2* (A) and *Cyp26b1* (C) in pairs of UGRs from (**E**) male and (**F**) female embryos at 12.0 dpc.

We tested whether this system might underpin the oogenesis-spermatogenesis dichotomy by using urogenital ridge (UGR, mesonephros plus gonad complex) organ cultures and a variety of molecular markers of meiotic progression. These included *Scp3* (*Sycp3*), which encodes a component of the synaptonemal complex, and *Dmc1* (*Dmc1h*), which encodes a meiosis-specific recombinase, both robust markers of meiotic prophase (*13*, *14*). Addition of all-*trans* RA to male UGR cultures induced the premeiotic marker *Stra8* (*15*) and expression of *Scp3* and *Dmc1*, as measured by quantitative RT-PCR (Fig. 3A). Moreover, RA treatment suppressed the pluripotency marker *Oct4* (*Pou5f1*) (Fig. 3A) (*16*). In all RT-PCR experiments, results were normalized against expression levels of *mouse vasa homolog* (*Mvh*) (*17*), a marker of germ cells, to control for germ cell number. We also implanted RA-soaked beads into UGRs cultured on agar; in situ hybridization confirmed up-regulation of *Stra8* in gonads of both sexes (Fig. 3B). Hence, we conclude that exogenous RA stimulates the entry of germ cells into meiosis. This is likely to be a direct effect on germ cells, because we and others have found that RA receptors (RARs) and retinoid X receptors (RXRs) are expressed by germ cells at the relevant stages of development (*18*–*20*) (fig. S2).

To test the requirement for endogenous RA signaling, we exposed cultured gonads to the RA receptor antagonist AGN193109 (*21*). When female UGRs were cultured in the presence of AGN193109, the expression of *Stra8*, *Scp3*, and *Dmc1* was substantially decreased (Fig. 3, C and D) as was the number of meiotic chromosome figures in histological sections (Fig. 3E). Exposure to the antagonist also prevented the decrease in *Oct4* expression normally observed in fetal ovaries (Fig. 3C). These results confirm that RA provides the normal, endogenous signal for entry into meiosis in the fetal ovary.

Is CYP26B1 the meiosis-inhibiting factor in males? To address this question, we first exposed gonadal organ cultures to the cytochrome P450 inhibitor ketoconazole (*6*). In testis cultures, expression of *Scp3* and *Dmc1* increased to levels normally seen in ovary cultures, whereas expression of *Oct4* decreased (Fig. 4A). The effect on *Scp3* expression was confirmed by in situ hybridization (Fig. 4B). Moreover, meiotic figures were abundant among germ cells in treated testes but not found in untreated testes (Fig. 4C). The identity of CYP26B1 as the meiosis-inhibiting substance in males was confirmed in vivo by analysis of *Cyp26b1*-null mouse embryos (*7*). In these embryos, *Stra8* and *Scp3* were strongly up-regulated in XY gonads relative to wild-type and heterozygous XY littermate controls (Fig. 4, D and E). Interestingly, meiosis progressed earlier than normal in *Cyp26b1*-knockout ovaries (Fig. 4F). Therefore, early expression of *Cyp26b1* in female genital ridges (Fig. 1A) may ensure that germ cells in the female do not enter meiosis prematurely, given that *Aldh1a2* is expressed in mesonephroi of both sexes from as early as 10.5 dpc (Fig. 2C).

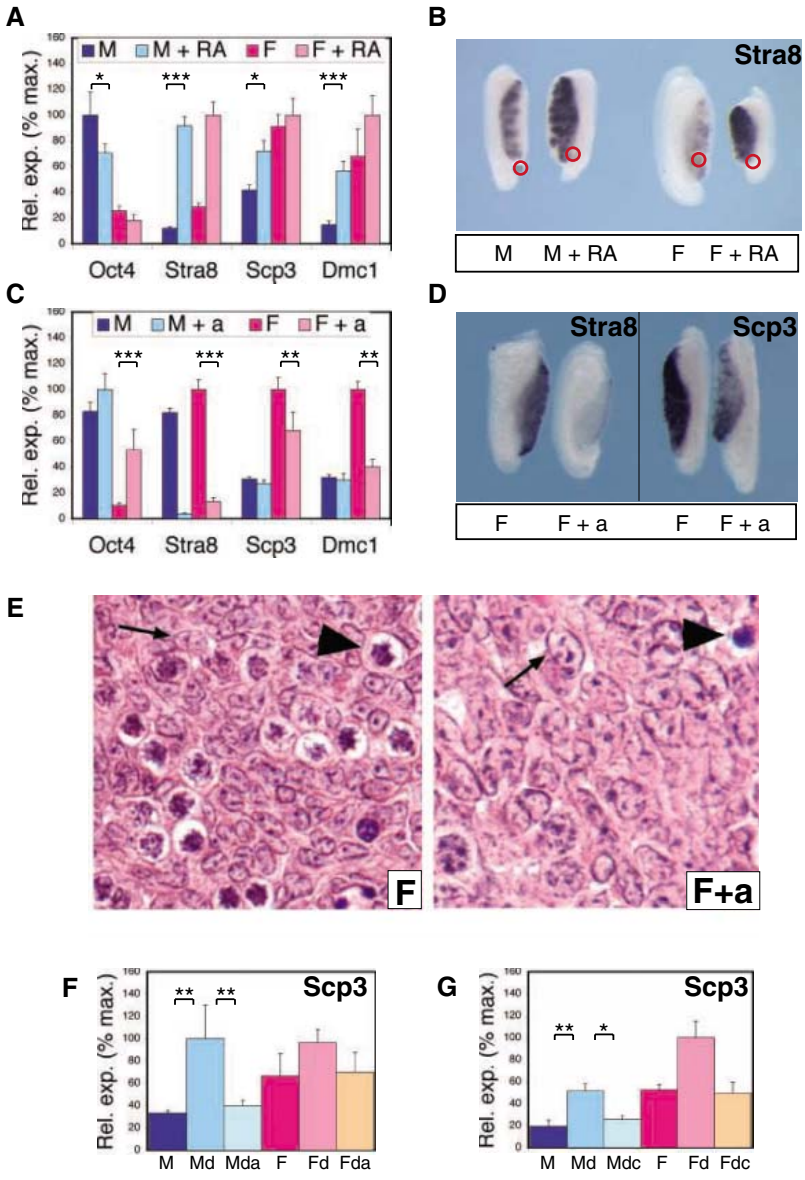


**Fig. 3.** Requirement for RA signaling in meiotic induction. Quantitative RT-PCR analysis of meiotic marker expression in UGR tissues treated with (**A**) all-trans RA or (**C**) RAR antagonist AGN193109. (**B**) In situ hybridization analysis of *Stra8* expression in UGRs after implantation with a bead (circled) soaked in all-trans RA. The samples shown were explanted at 12.5 dpc and cultured for 48 hours, which may explain the expression of *Stra8* in the control bead–implanted testis samples (fig. S3). (**D**) In situ hybridization analysis of *Stra8* and *Scp3* on female UGRs cultured with (F+A) or without (F) AGN193109. (**E**) Meiotic and nonmeiotic germ cells in 11.5 dpc–explanted plus 72 hour–cultured UGRs. Arrowheads, representative germ cells in meiotic prophase; arrow, representative nonmeiotic germ cells. Quantitation of *Scp3* expression after UGR dissociation-reassociation and culture with or without (**F**) AGN193109 or (**G**) citral. Bar graphs show the expression normalized to *Mvh*; error bars represent one standard deviation ($n = 3$). For (A) and (C), normalization to 18s rRNA was also carried out, with similar results (fig. S4). Expression is shown relative to maximal expression for the gene in question. Asterisks highlight the pertinent comparisons and indicate level of statistical significance (one asterisk, $P < 0.05$; two asterisks, $P < 0.01$; and three asterisks, $P < 0.0001$). a, AGN193109; d, dissociated-reaggregated; c, citral.

In previous studies (*2*), when 11.5-dpc male UGR cells were dissociated then reaggregated and cultured, germ cells entered meiosis, and it was postulated that destruction of the gonad architecture might disrupt produc-

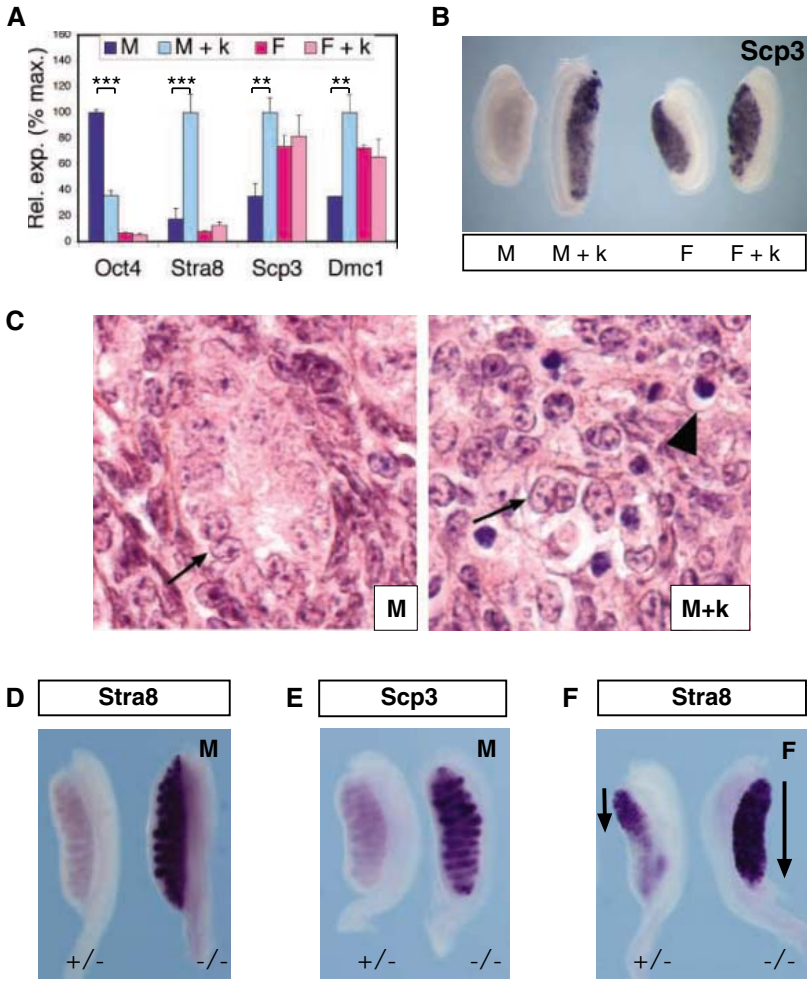


**Fig. 4.** CYP26B1 is the meiosis-inhibiting factor in male embryonic gonads. (**A**) Quantitative RT-PCR analysis of meiotic marker expression in UGR tissues treated with the P450 antagonist ketoconazole. (**B**) In situ hybridization analysis of *Scp3* expression in UGRs cultured with ketoconazole. (**C**) Meiotic and nonmeiotic germ cells in 11.5 dpc–explanted plus 72 hour–cultured UGRs. Details as in Fig. 3 legend; k, ketoconazole. (**D**) *Stra8* and (**E**) *Scp3* are strongly up-regulated in 13.5-dpc gonads of male embryos null for *Cyp26b1* (−/−) relative to heterozygous littermates (+/−). (**F**) *Stra8* is expressed in an expanded domain in 13.5-dpc gonads of female embryos null for *Cyp26b1*. Because meiosis occurs in an anterior-to-posterior wave in the developing ovary (*14*, *22*, *23*), meiosis is evidently more advanced in the knockout ovary (arrows). For each probe, four samples were analyzed for each genotype and representative samples are shown.

tion or activity of the meiosis-inhibitory factor. Our present data suggest that this treatment would place XY germ cells in close contact with RA-producing mesonephric cells and so induce meiosis. We tested this hypothesis by repeating these experiments in the presence of RAR antagonist AGN193109. Disassociation and reaggregation of untreated male UGRs at 11.5 dpc led to up-regulated expression of *Scp3* in XY germ cells (Fig. 3, F and G), consistent with published findings (*2*). However, when we included AGN193109 in aggregation cultures, *Scp3* was not induced (Fig. 3F). Similar results were obtained by using citral, an inhibitor of RA biosynthesis (*22*) (Fig. 3G). These results suggest that exposure of germ cells to RA-producing mesonephric cells was responsible for the induction of meiosis in the dissociated-reaggregated samples.

Induction of meiosis by RA released from the mesonephros is consistent with the anterior-to-posterior wave of meiotic progression in the fetal ovary (*15*, *23*, *24*). We observed very strong RA-induced gene expression in the mesonephric tubules (Fig. 2B), which are adjacent to the anterior pole of the gonad and connect the mesonephric duct with the gonad at 11.5 dpc (*25*). These tubules likely allow influx of RA, or RA-producing cells, to the gonad, resulting in earlier entry into meiosis anteriorly. This hypothesis is supported by transplantation and co-culture studies suggesting that the ovarian rete (the region containing tubules connecting mesonephros and ovary) produces a diffusible meiosis-inducing substance (*26*, *27*). Furthermore, in ovotestes, those germ cells that enter meiosis tend to lie adjacent to the ovarian rete (*28*), and in fetal testes meiotic germ cells are occasionally observed at the anterior junction between testis and mesonephros (*23*). Our results also accord with observations that RA can accelerate entry of rat germ cells into meiotic prophase when treated in cell culture (*29*).

Recent observations (*9*) indicate that RA stimulates *Stra8* expression in germ cells of mouse fetal ovaries and suggest that cytochrome P450 activity, ascribed to peritubular myoid cells, antagonizes this effect. Our experiments show that (i) RA induces full-scale meiosis, as judged by chromosomal condensation and molecular markers that directly correlate with meiotic prophase; (ii) the mesonephric duct and tubules are the source of RA in the urogenital system; (iii) high levels of RA are present in the fetal ovary but not the testis; (iv) *Cyp26b1* is expressed in Sertoli cells of the developing testis; and (v) CYP26B1 holds the key to preventing oogenesis in males by retarding meiosis in vivo. Although the existence of a meiosis-inhibiting factor was postulated some years ago (*2*), our studies reveal that this factor this is not a secreted signaling molecule as predicted but instead an enzyme of retinoid metabolism. Our findings may be applicable to modulating human or animal fertility in vivo and production of functional gametes from germline stem cells in vitro.

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# International Careers Report: Denmark

## Building on Tradition

**The Danish government has embarked on a plan to enhance its already strong profile in global life science. Denmark's Prime Minister and others involved in the process explain the plan's main ingredients: encouraging the academic, clinical, and industrial sectors to collaborate for the benefit of patients and to develop strategies for attracting top-notch life scientists from abroad.** BY PETER GWYNNE

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In recent years, Denmark has emerged as a major global player in life science research and commerce. The Scandinavian nation has a century-old history of developing pharmaceutical companies, an equally long tradition of conducting clinical trials, and a decade's worth of experience in creating biotechnology firms. Add to that the presence of a handful of authentically global pharmaceutical companies, most notably Novo Nordisk which specializes in treatments for diabetes, and it's clear why the country has gained its high profile.

Now, the Danish government wants to cement that global position and even improve it. To expand the country's appeal to investors and researchers in the international life science community, the government has set out a strategy based on the recommendations of the Globalization Council — a unique public council with five ministers and representatives from research communities, universities, industry, unions, employers, and others among its members. "My vision is a society that gives every CONTINUED »

# International Careers Report: Denmark

ANDERS FOGH RASMUSSEN
PRIME MINISTER OF DENMARK

HELGE SANDER
MINISTER OF SCIENCE

individual the opportunity to make a success of his or her life," says Prime Minister Anders Fogh Rasmussen, who chairs the council. "Therefore my government's goal is that Denmark becomes a global leader regarding sustainable economic growth development into knowledge society and dynamic entrepreneurship."

Based on the recommendations of the Globalization Council, the Danish Government has proposed a strategy that includes more than 300 initiatives, which extend well beyond life science or, indeed, science as a whole. Life science plays a key role in the strategy. Indeed, Minister of Science, Technology and Innovation Helge Sander notes that "the intention is to solidify Denmark's place on the world stage of breakthrough research in the life sciences." The effort will involve all sectors of Danish life science: universities, hospitals, and industry. Indeed, the strategy strongly emphasizes collaboration among those sectors. "To a high degree, challenges and inspiration come from collaboration with scientists from other sectors, such as private industry and other types of research institutions or from interdisciplinary settings," Sander continues. "We know that some of the best research environments in Denmark are also the environments with a high level of external funding and collaboration with external partners."

## Strong Infrastructure

The government has a strong infrastructure from which it can launch Denmark's expanded global presence. The country ranks first in the world in terms of biotechnology patents per member of the population, second in Europe in the European Union's best performance index of biotechnology innovation, and third in Europe in the absolute size of its drug development pipeline.

It all started with beer and pork. "The Carlsberg brewery and laboratory was instrumental in creating a research industry through microbiology 150 years ago," explains Lauritz Holm-Nielsen, rector of the University of Aarhus. "And the industry side of the equation probably stems from our strong agricultural background. To produce cheese, you need enzymes. We got into enzymes early and into insulin because we had a large availability of pork." Børge Diderichsen, vice president of corporate research affairs at Novo Nordisk, agrees. "We developed considerable competencies in agriculture-related industries," he says. "You can draw a line from barley to pigs to insulin and Novo Nordisk."

Those developments also instilled a culture of collaboration among Danish life scientists in academe and industry. "The starting point was strong, creative researchers who saw commercial opportunities in the medical discoveries being made in the 1920s," says Claus Bræstrup, president and CEO of pharmaceutical firm H. Lundbeck. "At one point, Danish politicians realized the value that the two sectors comprise together and laid down framework conditions that allow life science contributions in both the public and private sectors to continue to develop and grow." Collaboration also plays a key role in Denmark's academic research community. "There is an established tradition for close relationships between the basic and clinical sciences," explains Ralf Hemmingsen, rector of the University of Copenhagen. "The collaborative closeness between the basic science and clinical sciences and the industry and public health/epidemiology part of it has enhanced multidisciplinary projects."

CLAUS BRÆSTRUP

## Building on the Foundations

Jens Oddershede, rector of the University of Southern Denmark, points out another key factor in Denmark's development of life science: a long history of precise medical record-keeping. "We're very well organized," he says. "It's easy to trace our ancestors and place our genes. That's important for many studies in life science and clinical science."

Building on those 19th and 20th century foundations, modern Denmark has much to offer the world of life science. "The most important thing is the high educational level in Denmark," says Henrik Ditzel, professor and center coordinator of the Medical Biotechnology Center (MBC), part of the University of Southern Denmark and associated with Odense University Hospital. "Although Danes pretend to be very relaxed, they are very ambitious." Elisabeth Manford, director of Denmark's national investment promotional agency Invest in Denmark, agrees. "Great emphasis is placed on lifelong education and the general education system enjoys high priority," she says. "The result is a well-educated population with a high proportion of university graduates. Every year we turn out 400 Ph.D.s and 5,500 Master's graduates with life science–related degrees."

The collaborative ambience helps. "It is critically important," says Torben Greve, pro-rector for research at The Royal Veterinary and Agricultural University. "Training of young scientists is highly facilitated through such collaboration." It has also led to success in business. "There is very close collaboration between the industry and academic institutions, through economic contributions, joint projects, research academies, training of students in industry, and consulting boards," says Tore Duvold, vice president for drug discovery at LEO Pharma. "Several collaborations have resulted in the establishment of new companies based on innovative concepts."

The small size of both the country and its population (of roughly 5.5 million) also contributes to its strength in life CONTINUED »

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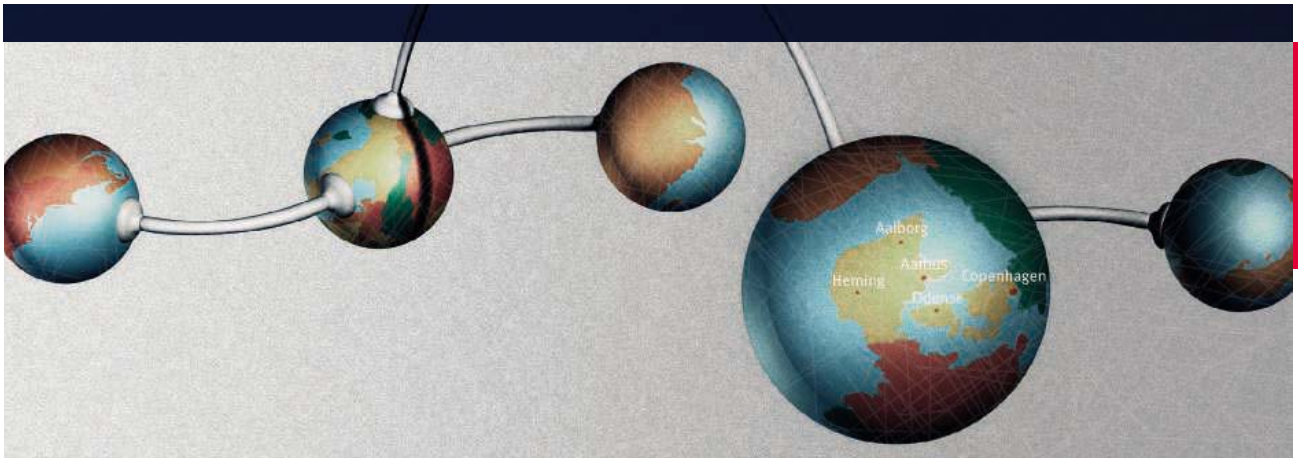


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# International Careers Report: Denmark

science. "Everybody knows everybody in a small country like Denmark," says Kristian Stubkjær, dean of research at the Technical University of Denmark (DTU). "It's easier to organize collaborations."

HASSE FERROLD

BØRGE DIDERICHSEN

## Concentration of Institutions

Collaborations also benefit from the concentration of research institutions. "Here in the Copenhagen region we have the medical school, the Pharmaceutical University, the Royal Veterinary School, and the Technical University. That's a very prosperous group within a six mile radius," Hemmingsen says. Manford makes a similar point in a larger context. "In an area no bigger than Silicon Valley we have eight leading universities, six university hospitals, more than 140 biotech companies, and 18,000 people working in the industry," she says.

The collaborative instinct extends across national borders. A six-year-old bridge over the Øresund, the stretch of water between Denmark and Sweden, links universities, research institutions, and life science companies in greater Copenhagen and their southern Swedish counterparts in Malmö and Lund. The result is the Medicon Valley, a hub of life science that rivals conglomerations in other parts of Europe.

The University of Aarhus's Holm-Nielsen points out another advantage of Danish geography. "Being small and agile, we can catch up relatively quickly – even if we start a little behind larger countries," he says.

Corporate governance has also helped to guarantee the prosperity of Danish life science. "We have a system in which some companies are protected by foundation structures, so that they can't be taken over," Diderichsen explains. "So we have been able to maintain a strong national research base in the life science industry while in Sweden almost all the well known pharmaceutical companies have disappeared or been merged."

The government has also played its part. In 2000, a new law gave Danish universities ownership of their researchers' inventions, thereby facilitating the universities' ability to make licensing deals with industry. As a result, Denmark has become an excellent country in which to do life science business – a fact that overseas firms have begun to recognize. "A strong position in R&D, together with a dynamic business environment and excellent framework conditions are contributing factors to why international companies like GE Healthcare, Johnson & Johnson, Procter & Gamble, GlaxoSmithKline, Merck, Aeras, Ferring, and Biogen IDEC have chosen to set up R&D centers or production facilities in Denmark," Manford says.

## Present and Future Initiatives

Science, Technology and Innovation Minister Sander sums up the current situation. "Over the last few years, a range of initiatives has been taken to improve the framework for life science. They include strategic research programs in biotechnology, incubators, and new science parks," he says. "We have a modern R&D framework, with new legislation governing the universities. This has given the universities more freedom with regard to self-governance. More emphasis has been placed on strategic research with the establishment of a specific research council for strategic results as well as the establishment of a national high-technology foundation."

HENRIK DITZEL

Given all those advantages, why does the government want to create a new global strategy? "International competition within R&D is getting strong and stronger," Sander says. "If we're not constantly seeking to improve our international standing in R&D, we may well fall behind. We must not get complacent and believe that our life science, as well as other fields, will continue to be successful." The strategy has a business objective as well. "Denmark consistently ranks among the best business environments in the world," Prime Minister Rasmussen explains. "But because of our small size, this fact is not very well known in the international community. Consequently, during the next few years we will increase our efforts to inform about the superior business opportunities offered to foreign investors in Denmark."

The government has proposed the strategy to do just that. "It is not only within R&D we are drawing up a globalization strategy," Sander explains. "Primary education must be improved; teachers' qualifications must be improved. Further education, R&D, and innovation are all areas where the government has a set of strategic initiatives to face the challenges of globalization. But it is of major importance that we are placing quality at the top of the agenda."

## Four Segments of Strategy

Four segments of the proposed strategy have particular relevance to life science:

- By 2010, public funding for R&D will amount to 1 percent of GDP, in contrast to the present figure of 0.75 percent. And overall, public and private funding of R&D will reach 3 percent of GDP. This implies substantial growth, achieved largely through the internationalization of Danish science.
- The model for funding academic research will undergo a significant change. The government will base its allocation of funds for basic research in universities on an overall evaluation of the level of quality and the goals presented in the development contracts. Universities will be assessed on their teaching, research, and knowledge proliferation. Those institutions that do best in the evaluation by international experts will receive relatively more funding.
- By 2010 at the latest, the government will base half of its support for research on open competition, as opposed to earmarks for specific institutions. Today, winners of open competition receive just one-third of the share of public R&D expenditure.
- The numbers of Ph.D. scholarships and industrial Ph.D.s will double. Ph.D. scholarships in natural sciences, technical sciences, and health sciences will gain particular benefit from this segment of the strategy.

The strategy focuses on scientists of the future as well as the present. "The government wants to improve the general CONTINUED »

Ministry of Science
Technology and Innovation

# Denmark – An active partner in the international knowledge society

Denmark has an open knowledge-based economy which for years has been attractive to foreign investment. In May, The Economist Intelligence Unit will publish their global outlook once again marking Denmark´s leading global position, with a comfortable margin, as the world´s most attractive country for foreign investment in the first decade of the 21st century.

But in Denmark we know that success cannot be taken for granted. Therefore the Danish Government has set a goal for the Danish society: Denmark is to be one of the best countries in the world to live and work in – also in 10 and 20 years time. Denmark is to be a country with a global outlook playing an active role in the international community; a country where everyone takes part in innovation and has a share in progress and prosperity; a country that is open to new ideas, people, and interaction with other countries and cultures. Denmark is to be an active and visionary partner in the international knowledge society.

## New Globalisation Strategy

The Government is now presenting its strategy for Denmark in a globalised world. The strategy contains more than 300 initiatives and entails reforms of the fields of education and research as well as notable improvements in the framework conditions for growth and innovation throughout the Danish society.

The Globalisation Strategy builds on a number of structural reforms in Danish research that have already been implemented, and we have strengthened the interaction between the business sector and the research and educational institutions.

It is a strategy that places quality and internationalisation at the top of the agenda. The funding for research will be increased significantly, and the number of grants for PhD students and Industrial PhDs will be doubled.

## Denmark welcomes international students, researchers and enterprises

We have a dynamic and knowledge-oriented business community and academic standards are high, measured for example by the number of publications and citations in internationally recognised scientific journals. A special 25 % tax scheme is providing favourable conditions for foreign key employees and researchers.

Denmark is a well-functioning democratic society with a high quality of life. The welfare system ensures equal rights and access to public services for all. Denmark is also characterised by gender equality, good public health, low crime, a clean environment as well as an active business- and cultural life and high-quality research and development environments.

*Come to Denmark and you will not regret it!*

**Helge Sander**
*Minister for Science, Technology & Innovation*

**Further reading:**

www.vtu.dk
www.denmark.dk
www.workindenmark.dk

# International Careers Report: Denmark

international outlook and understanding among students in Denmark," Sander says. "Danish students will be encouraged to take parts of their education abroad and the Danish educational system will be made more attractive to international students. These initiatives are part of the government's aim to ensure that new generations of the Danish work force are better prepared to face the challenges of globalization. But it should certainly also make them more attractive to employment in international companies within Denmark."

Rasmussen summarizes the strategy's goals. "We will implement methods in order to better utilize and transform R&D into new commercial technologies, products, and services," he says. "We will strive to create the best business environment in the world so that entrepreneurs can grow and compete."

Denmark's life scientists have welcomed the strategy. But they have one small concern. "There's enormous encouragement for us to do industrial-style applied research, which might take away from basic research over the long term," MBC's Ditzel warns. According to Stubkjær of DTU, industry echoes that sentiment. "The life science/pharmaceutical industry wants universities to do basic research, not to move into applications," he declares.


TORE DUVOLD

## Setting Up the Framework

Having announced its strategy for globalization, the government has begun to organize a framework that will enable it to flourish. That will involve extensive collaboration in the life science sector. "Different organizations, institutions, and companies will be collaborating on the implementation of specific initiatives," Sander says. "This could apply to the development of educational projects or formal traineeships in private companies. The Danish life science sector could play a major role with regard to this."

As it happens, most academic and industrial life science organizations have already started to set up collaborative projects and to look overseas for ideas and staff. A typical example is the work of the Danish Research Coordination Committee. "It is an attempt to put all important partners in research – the research councils, universities, and national laboratories – on the same committee," says the University of Southern Denmark's Oddershede, who formerly chaired the institution. "The idea is that those who give out the money and those who get it are presented with the same facts, so that we can design programs that will benefit both parties. The committee also has the main responsibility for funding graduate education. That's an excellent tool in directing research."

Visit **www.sciencecareers.org** and plan to attend upcoming meetings and job fairs that will help further your career.

The life science industry now plays a major part in encouraging academic research. One scheme involves government contributions as well. "The Danish government is supporting 70 new industrial Ph.D. fellowships per year," Novo Nordisk's Diderichsen says. "The company pays approximately half and the government half, and the Ph.D. students work both in a company and a university and are jointly supervised by scientists from both parties. This very successful program is also open to foreign Ph.D. students." Lundbeck's Bræstrup outlines the advantages of that scheme. "It builds bridges between industry and academia," he explains. "The Ph.D. students gain a broader understanding of their own opportunities and those of their profession, and program graduates are extremely well qualified as workers and bridge builders in both worlds."

Greve of The Royal Veterinary and Agricultural University points out that the programs have already begun to yield results. "These are seen in a number of publications, and in the increasing amount of patents of which universities have part ownership," he says.


ELISABETH MANFORD

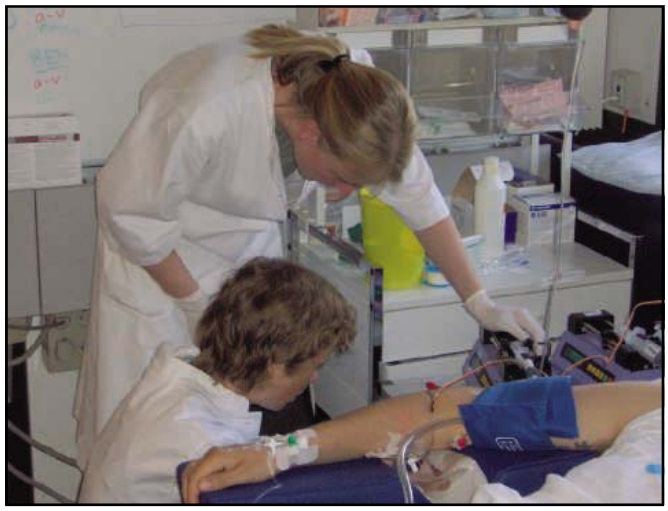
## Corporate Contributions

Individual firms make their own contributions to the support of academic research in the homeland and to spreading the word about Danish life science throughout the world. "We have major research and training collaborations with about 27 universities, hospitals, and research institutions globally, about half of them Danish institutions," Novo Nordisk's Diderichsen says. "These agreements are important because they mean money for the collaborating institutions and transfer of knowledge in both directions. This is also a joint confidence-building effort."

LEO Pharma has sponsored an incubator concept that helps individual scientists and university groups to develop promising research leads for therapeutic use. "We shared our knowledge and experience in drug development and gave the valuable consultancy needed to take the concepts further," Duvold says. In addition, he continues, "We regularly give economic support from our research foundation to fundamental research. We primarily focus on young and promising talents that are about to establish a group, a concept, or facilities. We also have direct collaborations in which we work actively with academic groups on developing new pharmacological models, elucidating biological mechanisms, and targeting proteins, technologies, or compounds with therapeutic potential."

Lundbeck owes its very existence to collaborative technology transfer and its continuing growth to an international outlook. "The company we see today was founded and created against a backdrop of a research discovery of a new medical product," Bræstrup recalls. "The discovery was put into practice, and has since resulted in growth and many jobs in Denmark – and also abroad. And it has laid the groundwork for our ability to continue our research and to develop new drugs to treat diseases of the central nervous system. We are also active participants in the development of the local research environment. For example, we are active board members for the various universities." CONTINUED »

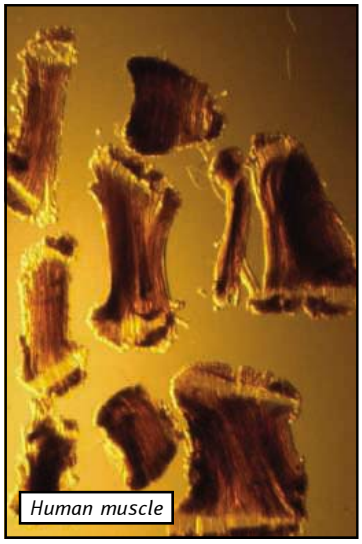
FACULTY OF HEALTH SCIENCES
UNIVERSITY OF COPENHAGEN



# *Be part of our exciting Campus life. You are most welcome,* says the Dean, Professor Ulla Wewer.

Since the University of Copenhagen was founded in 1479, the Faculty of Health Sciences has focused on the training of medical doctors. In the early 1990s this profile changed and the Faculty became multidisciplinary and now offers a wide range of study programs within health science. We take pride in developing new courses that meet the demands of today's and tomorrow's students.

The Faculty, with more than 4,000 undergraduate students, is a modern and technologically advanced research institution covering all disciplines of health sciences from biomedicine to public health and clinical medicine. Apart from medical and dental education, we offer study programs in human biology, public health, molecular biomedicine, medicine and technology, and nanotechnology. In addition more than 100 PhDs graduate from the Faculty every year.



*Human muscle*

The Faculty is located on a modern and dynamic campus in central Copenhagen, the capital of Denmark. The city has a vibrant cultural life and a wealth of options for spare time activities, and everybody speaks English.

## Come and study in a research environment

The Faculty wishes to stimulate its international profile by participating in research networks and collaborative projects, university alliances and student exchange programs with an international scope. For particularly talented students who take a specific interest in, and are motivated by, research we offer:

- Research-based study programs – link your studies to frontier knowledge
- Participation in exciting basic research – work next to the researcher in the laboratory
- Multidisciplinary student environment – meet new ways of thinking
- Elective courses – create your own program
- Small student groups under qualified supervision – the researcher is your mentor

## Tuition fees

Students from the European Economic Area (EEA) and exchange students from universities with an agreement with the University of Copenhagen can study at the Faculty of Heath Science **free of charge**. Other students pay a tuition fee. Students are accepted based on their previous educational achievements.

## Getting started

For more information on exchange contact your own university. However, the International Office at the University of Copenhagen (**www.ku.dk/international**) or the International Secretariat at the Faculty, att: Tina Gottlieb (**tig@adm.ku.dk**) will also be pleased to assist you.

## For further information on the study programs, please contact:

Human biology: Bodil Norrild **bodilnorrild@pai.ku.dk**
Public health: Lisbeth Ehlert Knudsen
**L.Knudsen@pubhealth.ku.dk**
Molecular biomedicine: Steen Dissing **sdissing@mfi.ku.dk**
Medicine and technology: Niels-Henrik Holstein-Rathlou
**niels@mfi.ku.dk**
Nanotechnology, including nanomedicine: Peter E. Nielsen
**Pen@ imbg.ku.dk**

## Research-focused study programs

### Human biology

Human biology is a two-year multidisciplinary study program within basic biological and biomedical subjects including tool subjects in natural sciences. It also includes long-term stays in clinical departments at the University Hospital. There is a strong emphasis on independent problem solving and methodology acquisition with individual guidance and hands-on laboratory exercises. The second year is devoted to an individual experimental research project under supervision.

Admittance requires a bachelor degree in, for example, natural sciences, exercise science, biochemistry or medicine.

The profile of the candidates from human biology is: in-depth biological knowledge and comprehension of human pathophysiology, understanding of research and development, and evaluation and counseling in relation to biological problems relevant to human health.

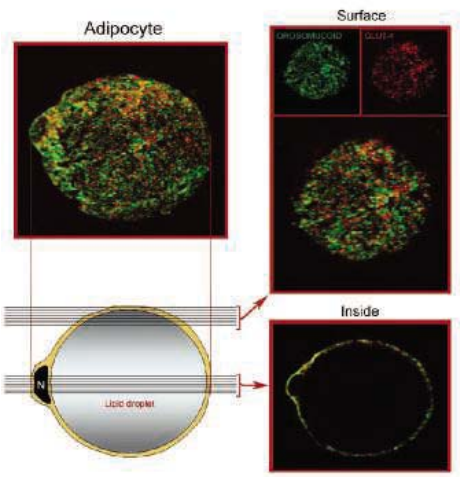
### Public health

The international study programme in public health sciences is interdisciplinary and encompasses elements from statistics, epidemiology, qualitative research methods, human biology, social sciences, international health, economics, prevention and health promotion. Much study time is devoted to projects. A thesis is written under supervision during the last term. The programme is based on close collaboration with research institutions working with public health issues.

The candidates are qualified for positions within research, public health administration, community health administration, and private enterprises.

### Molecular biomedicine

The explosive development in molecular biology and molecular biomedicine has prompted us, together with the Faculty of Science, to offer a new bachelor degree in molecular biomedicine. The students acquire specific competences in understanding the structure and function of normal and pathological situations at a molecular, cellular and physiological level. We focus particularly on advanced techniques and complex biological questions, and there is a strong focus on experimental hands-on work.




From September 2007, in conjunction with the Faculty of Science, we will also offer a masters degree in molecular biomedicine. The study program will include a number of elective courses and a thesis written during the last term under supervision.

### Medicine and technology

There is considerable need for academic professionals with a combined educational background in medical and biological sciences and classical biomedical engineering in many clinical departments, advanced imaging centers and radiotheraphy facilities, as well as in the biomedical engineering/biopharmaceutical industry. Together with the Technical University of Denmark we offer this study program to provide such professionals.

The study program is multidisciplinary, combining technical courses at the Technical University of Denmark with basic medical science at the Faculty. Courses can be combined in numerous ways, enabling the students to design their own program. Some examples are: biomechanics and biomaterials, imaging and radiation physics, and signal and model-based diagnostics.

The majority of the courses are taught in small groups, combining lectures with laboratory experiments using state-of-art technology.

### Nanotechnology, including nanomedicine

Together with the Faculty of Science the Faculty of Health Sciences offers a new study program in nanotechnology with nanobiological and nanomedical dimensions. The study program offers experimental courses in this area building on cutting-edge science, giving the students training and experience in designing, executing and interpreting scientific experiments.

# International Careers Report: Denmark


LAURITZ HOLM-NIELSEN

## Changing Attitudes

Academics' attitudes to collaborating with industry have shifted significantly in recent years. "There has been a big change in perception," MBC's Ditzel says. "University research used to be very separate from industry. Now, scientists have realized that they can benefit a lot from collaboration. Basically all the professors in my place have collaborations."

The University of Copenhagen has taken the initiative in working with industry. "We have increased our efforts in technology transfer services for researchers over the last five years," Hemmingsen reports. "We have, for example, established some industry professorships funded by the industry and appointed by the university, with a 50-50 time share between the firm and the faculty. Such professorships serve as paradigms for young researchers so they don't feel that, once they go into industry, they can't come back."

Universities have also started to set up formal academic collaborations. "There are current strong efforts to create synergy between our university, which is focused on food, and other Danish institutions focused on pharma and drugs," Greve says. "In fact we have just created a Danish Pharma Consortium, and we are trying to get a European technology platform in this field into the Copenhagen area." In addition to Greve's veterinary university, the consortium includes the University of Copenhagen, the Pharmaceutical University, and the Technical University. "Our consortium will collaborate with other organizations, such as research institutes, research hospitals, and the pharma industry," says DTU's Stubkjær.

The University of Copenhagen has taken a step further, by joining a worldwide Alliance of Research Universities. "It was inaugurated in January 2006," Hemmingsen says. "It includes strong institutions in the United States, such as Berkeley and Yale, as well as Oxford and Cambridge in the United Kingdom. We'll hopefully get exchanges of a few students, and some collaborations involving medical scientists."


JENS ODDERSHEDE

## International Announcements

Danish universities also have active efforts to recruit life science faculty members and graduate students from overseas. "Recruiting professors from abroad is taken very seriously by our university," Greve says. "All positions are announced internationally, and we try very hard to convince applicants that our university and Denmark represent the best place to stay."

Part of the recruitment process involves making academic institutions comfortable for individuals from different scientific cultures. Ditzel, who moved from the Scripps Research Institute in the United States, helped to set up the University of Southern Denmark's Medical Biotechnology Center with an American-style organization in which several professors head their own large research groups. "It was a very inspiring idea to have a fresh start with seven professors starting at the same time," he recalls. The MBC faculty team has a strong international flavor, with professors from Canada and Germany, as well as Denmark and the United States.


KRISTIAN STUBKJÆR

Since English has become the de facto lingua franca of life science, Danish universities use the language as a powerful stimulus for overseas recruitment. "All our meetings are in English," Ditzel says. "And our e-mails to each other are in English. We also have interest in helping people to adapt to the country. But you can get around without speaking the native tongue."

Other universities take a similar line. "We have recently negotiated an agreement with the government that includes more courses and whole degrees that can be taken in English," the University of Copenhagen's Hemmingsen says. "We are announcing positions for professors and postdocs in English and often in the international journals."

## Traction for English

The trend to teaching in English has the greatest traction at the graduate level. "The language is Danish at the undergraduate level, although we have some programs in English and we do everything we can to make the framework comfortable for foreign students," Holm-Nielsen of the University of Aarhus says. "At the graduate level, language is not an issue. We use the English language and original literature. We also have Danish language courses and good programs for spouses." At DTU, Stubkjær adds, "Most of our courses at the Master's level are given in English. In most of our departments the working language is English." Much the same is true of the University of Southern Denmark, which has faculty members from several European countries, Australia, former nations of the Soviet Union, and the United States. "We have offered a range of Master's programs in life science and other areas in English," Oddershede says. "The Ph.D. program ties up with the Master's program in English."

Some institutions offer a little extra help to acclimate newly arrived faculty to the mores of Danish science. "We have a two-year support scheme for new professors that provides direct economic support for their research until they get used to the Danish system of research councils," Stubkjær notes. That type of assistance complements a government incentive announced recently that charges foreign scientists tax at a rate of 25 percent of income – rather than the usual 55 percent to 60 percent – during their first three years in Denmark.

Those efforts, combined with the government's new strategy, promise to keep Denmark high in the global league of life science. "In the years to come, we will see the results of a range of life science initiatives," Minister Sander says. "In the Copenhagen area, a major bio-center is presently under construction. This center will include industry and academia, a science park, and world-class science environments. And the new, national high-technology foundation is to have a specific focus on biotechnology. I see a variety of potential for growth within Danish life science that could lead to world-class science within academia as well as industry."

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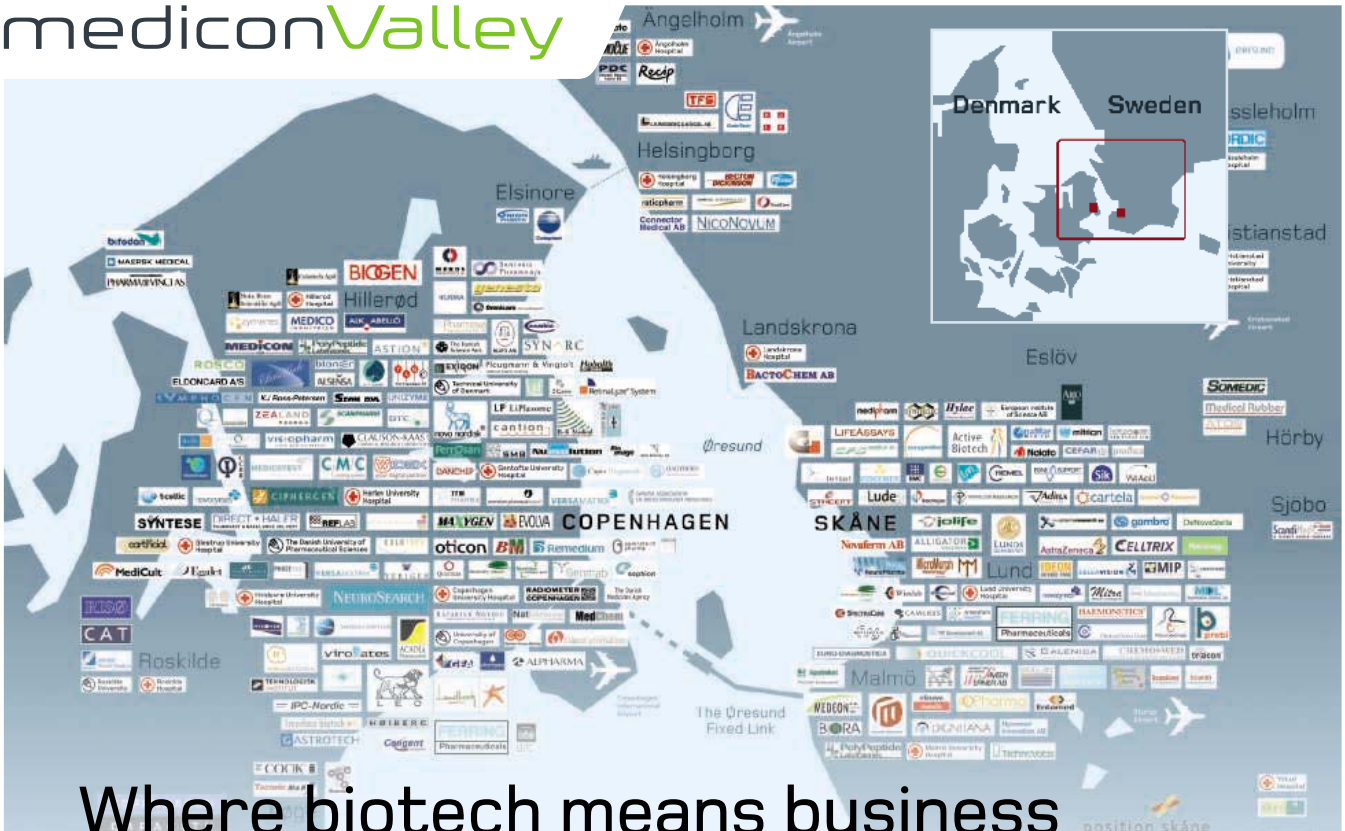
*A former science editor of* Newsweek, *Peter Gwynne (pgwynne767@aol.com) covers science and technology from his base on Cape Cod, Massachusetts, U.S.A.*

# mediconValley




# Where biotech means business

Copenhagen - the capital of Denmark - is **Scandinavian City of the Future** according to Financial Times' fDI Magazine, and **The country offering the best business conditions in the world** according to The Economist Intelligence Unit.

Copenhagen is also the centre of Medicon Valley, one of the strongest life science clusters in the world. This exciting Danish-Swedish cluster contains a concentration of pharmaceutical and biotech companies unmatched in Europe and boasts leading positions globally within some of the most commercially attractive disease areas.

## Business opportunities in Medicon Valley

Copenhagen Capacity is the official regional investment promotion agency. We can help your company in identifying and exploiting business opportunities in Medicon Valley. The agency offers free advice and assistance to foreign companies considering establishing activities in Medicon Valley.

**Medicon Valley at a glance**

- Approx. 3 million inhabitants
- 14 universities
- 140,000 university students
- 5,000 life science researchers
- 26 hospitals (11 university hospitals)
- 7 science parks
- 140 biotech companies
- 70 pharma companies
- 130 medtech companies
- 15 clinical research organisations
- 30 investors
- 250 service providers and others

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COPENHAGEN CAPACITY

# Six Postdoctoral positions at iNANO, Denmark

The Interdisciplinary Nanoscience Center (iNANO) (www.inano.dk) is a major research and education center based at the University of Aarhus and Aalborg University. The center currently undertakes interdisciplinary research involving scientists from relevant areas in physics, chemistry, molecular biology, biology, engineering, and medicine. iNANO offers a dynamic, interdisciplinary research environment with many industrial, national and international collaborators.

At the iNANO center we currently have a number of open postdoctoral positions. The potential candidates should submit their applications as soon as possible, and no later than June 15th, 2006.

**AFM studies of biomolecules**: We are seeking a candidate with strong expertise in liquid phase atomic force microscopy (AFM) and in the synthesis of micro and nanostructures, who can contribute to interdisciplinary projects within the FP6 EU-project "NANOCUES" (**www.nanocues.org**)

**AFM and microcantilever studies of anti-biofouling surfaces**: The successful candidate should actively contribute to interdisciplinary projects focusing on the characterization and development of anti-biofouling surfaces. This includes a molecular-level investigation of bacterial adhesion mechanisms on chemically and/or topographically modified surfaces. Characterization tools include atomic force microscopy (AFM), scanning electron microscopy, fluorescence microscopy, and cantilever-based biosensors. Candidates with expertise in one or several of these techniques, bacterial adhesion, and/or a general interdisplinary background in biophysics, biochemistry or molecular biology are preferred.

**Surfaces of Nanononwovens**: The successful candidate should actively contribute to a joint collaboration between Fibertex A/S (**www.fibertex.com**) and iNANO with the aim of using nanotechnology approaches to modify non-woven polymer fibres that add functionality and can be used in a range of different application areas, such as in the medical, hygiene or industrial sector. Applicants should ideally have a background in chemistry/polymer with some knowledge of surface-related phenomena. Knowledge of modification and processing of polymer fibres would be a distinct advantage.

**DNP enhanced NMR studies of biological macromolecules:** We have an open three-year postdoctoral position concerned with the development of theoretical descriptions and numerical simulations of DNP enhancement of NMR experiments as well as applications to membrane proteins. The ideal candidate has expertise in NMR relaxation, software programming (c-code, SIMPSON), and knowledge of DNP. The project is financed by the EU project Bio-DNP (**www.bio-dnp.org; www.inspin.dk**).

**Solid-State NMR Spectroscopy of Membrane Proteins and Fibrils:** We are seeking candidates with strong expertise in biological solid-state NMR spectroscopy; this includes NMR experiments, sample preparation, and data interpretation in the given order. The successful candidate will be centrally involved in the study of membrane proteins, fibril and extracellular matrix proteins according to the mission of the Center for Insoluble Protein Structures (**www.inspin.dk**).

**Biophysical characterization of fibrillating proteins and peptides.** The successful candidate will work at the University of Aalborg (Prof. Daniel Otzen) and in close contact with the solid-state NMR group at University of Aarhus to provide suitable samples for biophysical and spectroscopic studies. Practical experience with protein characterization (spectroscopic techniques, *e.g.* fluorescence and circular dichroism to measure stability and folding kinetics) is required. In addition, the ability to work with large amounts of data is a distinct advantage.

For further information, please contact the iNANO Director, Prof., D.Sc. Flemming Besenbacher (fbe@inano.dk) or Prof. Niels Chr. Nielsen (ncn@inano.dk). Potential candidates should send their CV, full publication list, and a short description of qualifications, research plan, and research network to **inano@inano.dk**

Ministry of Science
Technology and Innovation

workindenmark.dk

# International career
# – Working and living in Denmark

We want Denmark to become one of the world's leading countries when it comes to science, innovation, entrepreneurship and development. Denmark has a long tradition of facilitating an innovative, safe and creative environment.

In Denmark we offer excellent working conditions, modern conveniences and technical equipment of high standards. Furthermore we have one of the world's best social security systems and favourable taxes for foreign researchers who work and live in Denmark.

## Denmark wants to be an international leader in science and innovation - do you?

**www.workindenmark.dk**

Positions @ NIH
THE NATIONAL INSTITUTES OF HEALTH

## Postdoctoral Fellowship in Proteomics of Innate Immunity Signaling

Applications are invited for a fellowship in proteomic analysis of innate immunity signaling pathways. Projects will use mass spectroscopy and ancillary techniques to target protein-protein interactions, post-translational modifications, and protein discovery in the Toll Like Receptor Pathways, and to biologically validate findings.

Applicants should possess a Ph.D. degree in Molecular Biology, Cellular Biology, Biochemistry, Immunology, Pharmacology, or a related field, and have no more than five years of relevant postdoctoral experience. Hands-on experience in mass spectroscopic techniques is very strongly preferred; an extensive background in biology is not necessary. Salary will be commensurate with experience according to NIH guidelines.

For additional information about this position, contact **Dale Kersey at kerseyd2@niehs.nih.gov** and the inquiry will be forwarded to the appropriate individual for response. For prompt consideration, send or e-mail cover letter including a summary of relevant experience, CV including list of publications in peer-reviewed journals, and the names/phone numbers of 3 people who could provide letters of reference to: **Dale Kersey (HNV06-04), National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, 111 T.W. Alexander Drive, Rall Building, Maildrop A2-06, Room A207, Research Triangle Park, NC 27709 or kerseyd2@niehs.nih.gov**.

## Scientific Review Administrator
## National Heart, Lung and Blood Institute

The National Heart, Lung, and Blood Institute (NHLBI), a major research component of the National Institutes of Health (NIH), Department of Health and Human Services (DHHS), is seeking two Scientific Review Administrators for the Review Branch, Division of Extramural Affairs. Scientific Review Administrators organize and manage the comprehensive scientific and technical merit review of grant applications and contract proposals through interaction with established scientists in a variety of fields. Scientific Review Administrators are responsible for assuring the fairness and consistency of the review process, and for providing technical guidance to applicants, reviewers, and Institute staff.

Qualifications: Individuals with a Ph.D. or doctoral degree equivalent, and a scientific background in disciplines relevant to heart, lung, blood, or sleep disease research, are encouraged to apply. Experience in grant preparation and in the peer review process is desirable. For the basic qualification requirements, please refer to the NIH guidance for Health Scientist Administrators at http://www.nhlbi.nih.gov/about/jobs/hsaguide.htm. U.S. citizenship is required.

Salary: The current salary range is $ 65,048 to $118,828. In addition, a recruitment bonus may also be considered. Position requirements and detailed application procedures are provided on vacancy announcement **NHLBI-06-123471**, which can be obtained by accessing **http://WWW.USAJOBS.GOV**.

How to Apply: Please view the above USAJobs website for full vacancy announcement. This vacancy requires the submission of narrative Knowledge, Skills and Abilities (KSAs) as part of the application process. You may apply online at the above website or submit a Standard Form 171, Application for Federal Employment; OF-612, Optional Application for Federal Employment; current curriculum vitae/bibliography or other format to: **National Heart, Lung, and Blood Institute, Human Resources Branch G, 2115 E. Jefferson Street, Room 1-M100, Bethesda, Maryland 20892; Attn: Leanna Lomax**. All applications must be postmarked by **06/20/2006**. For additional information contact **Leanna Lomax at (301) 402-8032**.

## Staff Clinician - Endocrinology

The National Center for Complementary and Alternative Medicine (NCCAM) seeks outstanding candidates for a Staff Clinician position in Endocrinology in its Laboratory of Clinical Investigation (LCI) on the National Institutes of Health campus in Bethesda, Maryland. Applicants must possess an M.D. or D.O. degree, be licensed to practice medicine within the United States, have substantial clinical and research training in endocrinology, and be American Board of Internal Medicine (ABIM) eligible or certified in endocrinology and metabolism.

The successful candidate will be expected to contribute to existing clinical protocols in the Endocrine Section, LCI, and to conceptualize, write, and conduct new patient-oriented research studies designed to assess the potential efficacy, safety, and mechanisms of action of selected, complementary and alternative (CAM) modalities. A specific research interest and experience in the areas of neuroendocrinology, aging and/or stress biology, and a strong publication record in phase I, II and/or III clinical trials, are required.

The LCI, NCCAM provides state-of-the-art research facilities in the intramural program at NIH in addition to a collegial and nurturing working environment. The extensive clinical research facilities of the NIH Clinical Center will be made available to the successful applicant. Salary and benefits will be commensurate with experience. Applications from women and minorities are encouraged.

Please email your CV, bibliography, the names of three references, and a one page cover letter stating your scientific interests and experience to:

**nccamjobs@mail.nih.gov**
**Subject Line: Staff Clinician-Endo Search**

Candidates needing reasonable accommodation may fax application materials to **301-402-4741.**

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NIAAA
National Institute on Alcohol Abuse and Alcoholism

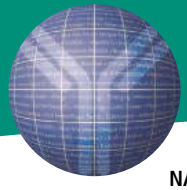
## Tenure Track Position in Nutritional Neuroscience

The National Institute on Alcohol Abuse and Alcoholism, a major research component of the National Institutes of Health and the Department of Health and Human Services, is recruiting for an outstanding scientist to lead a research program in the Laboratory of Membrane Biochemistry & Biophysics, Section of Nutritional Neuroscience (http://www.niaaa.nih.gov/ResearchInformation/IntramuralResearch/AboutDICBR /LMBB/ ), headed by Dr. Norman Salem, Jr. The Laboratory is involved in both basic and clinical studies of the role of polyunsaturated fatty acids in nervous system function. This will be a tenure-track position with a budget and personnel adequate to ensure a successful research effort. The individual selected for this position will conduct clinical, translational and laboratory research involving modifiable factors such as diet that modulate nervous system structure and function.

The successful individual will possess an M.D. or Ph.D. degree and have interest in neurological or psychiatric diseases and possess leadership/ managerial skills.

Salary is commensurate with research accomplishments and experience, and a full package of benefits (including retirement, health, life and long term care insurance, Thrift Savings Plan participation, etc.) is available. Applications should be received by **July 1, 2006**.

Interested candidates should submit their CV, list of publications, a brief statement of research interests and have three letters of reference submitted to **Brenda Robertson at 5635 Fishers Lane, Room 3049, Bethesda, MD 20892-9304 or broberts@mail.nih.gov**. For non-US citizens, ECFMG certification is required for those with an M.D. degree. Applications from women, minorities, and persons with disabilities are strongly encouraged.



## Health Research in a Changing World

Fighting Diseases and Improving Lives

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**2006 NIAID RESEARCH CONFERENCE**
**June 24-30, 2006, Opatija, Croatia**

The National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health invites you to participate in the 2006 NIAID Research Conference to be held in Opatija, Croatia on June 24-30, 2006. The program of the **2006 NIAID Research Conference** includes 2-day Satellite Symposium (June 24-25) open to all participants on "Grants Opportunities and Preparation" (grantsmanship, technology transfer, regulatory affairs, and FDA regulations with concurrent workshops), as well as training opportunities at NIH by the Fogarty International Center. The Scientific Program of the Conference (June 26-30) will include a wide range of sessions by invited speakers on topics reflecting the scientific activities of NIAID including global emerging and re-emerging infections. The 2006 NIAID Research Conference will also feature Plenary Lectures delivered by distinguished scientists, roundtable discussions on a wide variety of topics, and poster sessions open to all participants.

There will be NO registration fees for the conference. The Call for Abstracts submission and the Call for Poster submission will have a deadline of April 30, 2006.

**Complete information including conference registration will be available April 10, 2006 on the NIAID website (http://www3.niaid.nih.gov/program/croatia/)**

***DHHS and NIH are Equal Opportunity Employers***

# I want to explore.

## Neuroscientist, neurogenic inflammation

Mölndal, Sweden.

We are looking for an experienced molecular neuroscientist with expertise in the field of neurogenic inflammation with focus on the intestine. You will be
a key player in the strengthening of our activities on inflammatory bowel
disease, including identification of drug targets and establishment of functional experimental models for target validation and drug screening. You will act as scientific expert in the field and develop broad interactions with scientists within AstraZeneca and with academic partners. The ideal candidate will have a PhD and at least 5 years post doctoral experience in experimental neuroscience and inflammation. Experience from pharmaceutical R&D is an advantage.

For further information please contact Jan Mattsson,
phone: +46 708 46 76 77.

Please send your application and CV marked "71-11399 Neuroscientist, neurogenic inflammation" no later than 18th of May 2006 via www.astrazeneca.se. We will only handle applications received via our website.

www.astrazeneca.se

AstraZeneca
life inspiring ideas

# I want to influence change.

## Team Leader, Integrative Pharmacology

Mölndal, Sweden.

We are looking for an experienced scientist to lead and develop our team and the people working within the inflammatory bowel (IBD)
disease area. You will be accountable for the development of functional experimental models and will be involved in identifying new targets and treatment options within the IBD area. An important component will be to act in collaboration with scientists both within AstraZeneca and with academic partners. You must be a team player with strong skills in leadership.The ideal candidate will have a PhD in medical /biological sciences possibly in combination with an MD, and long-term experience from pharmaceutical R&D would be an advantage.

For further information please contact Per-Göran Gillberg,
phone: +46 708 46 74 53.

Please send your application and CV marked "71-11398 Team Leader, Integrative Pharmacology" no later than 18th of May 2006 via www.astrazeneca.se. We will only handle applications received via our website.

www.astrazeneca.se

AstraZeneca
life inspiring ideas

# UT-ORNL Governor's Chairs

The University of Tennessee in partnership with Oak Ridge National Laboratory is recruiting leading scientists to conduct research in the new **Joint Institute of Advanced Materials** with access to some of the most advanced scientific tools available. In addition to working in an exciting atmosphere of intellectual and academic freedom, the successful candidates would be living in one of the most beautiful areas in the country with easy access to miles of inland waterways, pristine state and national parks, diverse cultural opportunities and a unique mix of convenient urban and rural living settings.

**Find out more at http://www.tennessee.edu/governorschairs/**

## Governor's Chairs in the UT-ORNL Joint Institute for Advanced Materials

The State of Tennessee is investing funds to recruit and support approximately 20 exceptionally accomplished researchers who will have joint appointments as tenured professors at the University of Tennessee (UT) and distinguished scientists at Oak Ridge National Laboratory (ORNL). This Governor's Chair (GC) program seeks to catalyze the development of cutting edge research under the auspices of four joint institutes between the UT and ORNL: Biological Sciences, Computational Sciences, Neutron Sciences, and Advanced Materials. The GC appointments are 12 months, including an ongoing discretional research fund equal to the salary.

### The Joint Institute for Advanced Materials (JIAM).

JIAM will support research and education in interdisciplinary programs aimed at the discovery and utilization of new materials with interesting and applicable functionality. JIAM will work closely with the other three joint institutes.

The UT-ORNL environment nurtures a rich interdisciplinary community of researchers with common interest and collaborative projects. The UT-ORNL research enterprise also has more that $2 billion in investments in some of the world's most advanced research user facilities.

### There are immediate openings for JIAM GCs.

- **An experimentalist in Transport and Functionality in Complex and/or Nanostructured Materials.**
- **A theorist in the field of Modeling and Simulation of Nano-scale Materials.**
- **An experimentalist in the area of Controlled Synthesis and Directed Assembly.**

UT and ORNL research environment favors cross-disciplinary cutting-edge efforts that leverage special facilities in the physical and computations sciences. World-class research facilities include Leadership Class Computing, the Spallation Neutron Source, the upgraded High Flux Isotope Reactor, the newly opened Center for Nanophase Materials Sciences, the new JIAM building and associated instrumentation, and world-class electron microscope facilities.

Successful candidates will have an exceptional record of scientific productivity and accomplishment, as manifested, for example, in high-impact publications, scientific awards, and impressive citation numbers. Successful candidates will also have a demonstrated record of leading interdisciplinary or cross-disciplinary teams of researchers, and of developing substantial externally funded research programs.

APPLICATIONS: All applications should be submitted electronically to Jean Ponder (jponder@tennessee.edu) following the instructions associated with each search (see web site). Screening of applications will commence on June 1, 2006 and will continue until the positions are filled.

The University of Tennessee is an EEO/AA/Title VI/Title IX/Section504/ADA/ADEA institution in the provision of its education and employment programs and services. UT welcomes and honors people of all races, creeds, cultures, and sexual orientations and values intellectual curiosity, pursuit of knowledge, and academic freedom and integrity. ORNL, a multiprogram research facility managed by UT-Battelle, LLC, for the U.S. Department of Energy, is an equal opportunity employer committed to building and maintaining a diverse work force.

If you believe that you are of the quality we are looking for and can initiate new advanced materials programs at UT-ORNL, but do not fall into one of the three categories listed contact Ward Plummer (eplummer@utk.edu)

**Scientists and Engineers at ORNL and UT conduct basic and applied research and development to create scientific knowledge and technological solutions that strengthen the nation's global competitiveness; train the next generation scientists and engineers; increase the availability of clean, abundant energy; restore and protect the environment; and contribute to national security. UT and ORNL provide an environment that encourages collaborative research and development. UT-Battelle manages and operates ORNL.**

UT-BATTELLE

THE UNIVERSITY of TENNESSEE

OAK RIDGE National Laboratory

## MEDICAL OFFICERS AND INTERDISCIPLINARY SCIENTISTS PANDEMIC INFLUENZA VACCINE

**The Center for Biologics Evaluation and Research, Food and Drug Administration, Department of Health and Human Services** is searching for outstanding physicians and scientists to assist in the Center's Pandemic Influenza Vaccine initiative. Center staff conducts biomedical research to provide a strong scientific base for the regulation of blood and blood-related products, vaccines, allergenic products, and gene therapies according to statutory authorities in order to protect and enhance the public health. In conjunction with regulatory and research responsibilities, the Center statistically evaluates clinical and pre-clinical studies of human biological products and vaccines and epidemiologically evaluates post-marketing studies and adverse biological reactions.

The Pandemic Influenza Vaccine initiative is to protect critical workers and the US population in the anticipation of an influenza pandemic as quickly as possible through the establishment of a pandemic influenza vaccine manufacturing capacity. FDA has dramatically expanded its pandemic influenza program in the clinical, statistical, epidemiological, manufacturing and facilities areas this past year. FDA will be required to perform additional data reviews and facility inspections both for assuring adequate annual flu vaccine and in response to the potential for a pandemic.

**QUALIFICATIONS:**

- **Physicians:** Applicants must have an M.D. or equivalent degree from an accredited institution and additional research experience. Graduates of foreign medical schools must submit a copy of their ECFMG certificates.
- **Scientists:** (Other than M.D.) An advanced degree in one or more of the following disciplines is highly desirable: Biology, Microbiology, Chemistry, Biochemistry; Toxicology/Pharmacology, or Mathematical Statistician.

**CANDIDATES FOR CIVIL SERVICE OR COMMISSIONED CORPS APPOINTMENTS MUST BE U.S. CITIZENS. NON-U.S. CITIZENS MAY BE ELIGIBLE FOR SERVICE FELLOWSHIP APPOINTMENTS OR OTHER POST-DOCTORAL PROGRAMS.**

**SALARY:**

- **Physicians:** salaries range from Selected Federal White-Collar Pay Schedules, $97,213 - $114,882. In addition, physicians may also be eligible for a Physician's Comparability Allowance (PCA) of $4,000 to $24,000 per annum, or be appointed under Title 42 Excepted Service not to exceed $114,882. Salary and benefits are commensurate with education and experience. Positions may be filled by appointment in the US Public Health Service, Commissioned Corps.
- **Scientists:** salaries range from Selected Federal White-Collar Pay Schedules $52,468 - $114,882. Salary and level of responsibility are commensurate with education and experience.

**LOCATION:** CBER is actively recruiting applicants to fill positions located in Bethesda and Rockville, Maryland, involved in the regulation of biological and related products in support of the Pandemic Influenza Vaccine.

**HOW TO APPLY:** Applications are accepted and should indicate availability for employment. Interested candidates should submit a current Curriculum Vitae/Resume and cover letter to: **Food and Drug Administration, Center for Biologics Evaluation and Research; 1401 Rockville Pike, HFM-123, Rockville, MD 20852-1448; ATTENTION: Recruitment Coordinator**.

Additional information: **http://www.fda.gov/cber/inside/hirebkg.htm**.

*FDA IS AN EQUAL OPPORTUNITY EMPLOYER. SMOKE FREE ENVIRONMENT.*
**FDA provides reasonable accommodations to applicants/employees with disabilities.*

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PENNSTATE
University Park

## ASSISTANT PROFESSOR OF AVIAN BIOLOGY

The Pennsylvania State University College of Agricultural Sciences (**http://www.cas.psu.edu**) is one of the premier and largest agricultural colleges in the nation. In 2005, the College established a Reproductive Biology Initiative in order to enhance and expand collaborative and interdisciplinary research and teaching efforts in animal reproductive biology. As part of this Initiative, the Department of Poultry Science (**http://poultry.cas.psu.edu**) is seeking to fill a 36-week, tenure track faculty position in the area of avian reproductive biology.

The successful applicant will be expected to develop and maintain a productive, extramurally funded research program with a focus that could include, but is not limited to, infertility, basic mechanisms regulating fertility, gonadal function and production of gametes, hypothalamic-pituitary-gonadal axis, development of the embryo and reproductive tissues/organs, cryopreservation of gametes, or development of monosex populations. Individuals with research interests in stem cell or primordial germ cell biology and/or experience in the manipulation of egg proteins via transgenic technology (i.e., avian bioreactors) are also encouraged to apply, while applicants with experience in non-avian models will also be considered. The successful applicant will be expected to interact and collaborate with other faculty members with interests in reproductive biology or related disciplines, and numerous opportunities for such collaborations exist through rapidly growing, interdisciplinary research efforts in Penn State's Huck Institutes of the Life Sciences (**http://www.lsc.psu.edu**). Excellent core facilities exist for cell biology, genomics, proteomics and transgenic animal research while modern, AAALAC-approved facilities for poultry housing are available. The successful applicant is also expected to actively participate in undergraduate and graduate teaching in the areas of reproductive biology, endocrinology, avian biology, integrated animal biology, or animal physiology. Qualified applicants should possess a Ph.D. or equivalent degree with post-doctoral training in reproductive biology or related disciplines. Starting date is negotiable and competitive salary and benefit packages are available.

Applicants should submit a letter of application, curriculum vitae, separate statements addressing research and teaching interests including interdepartmental and interdisciplinary research collaborations and teaching philosophy, interests, and experience, respectively, and the names, addresses, telephone numbers and email addresses of at least three references to: **Dr. Ramesh Ramachandran, Chair, Faculty Search Committee, Department of Poultry Science, The Pennsylvania State University, 213 Henning Building, University Park, PA 16802**. E-mail: **RameshR@psu.edu**. Applications will be accepted until **June 15, 2006** or until a qualified candidate is identified.

*PENN STATE IS COMMITTED TO AFFIRMATIVE ACTION, EQUAL OPPORTUNITY AND THE DIVERSITY OF ITS WORK FORCE.*

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UCLA

The Division of Life Sciences at UCLA invites applications for a tenured faculty position at the level of Associate or Full Professor. Applicants should have a record of creative and significant teaching, research and mentoring of underrepresented students in any area of biological science. Appointment could be in one or more departments within the Life Sciences. The successful candidate will be expected to direct the UCLA-NIH Minority Access to Research Careers (MARC) Program, participate in undergraduate and graduate teaching, and establish a vigorous, extramurally funded research program.

Interested applicants should submit a curriculum vitae with a description of research plans, reprints or key preprints of publications, details of experience with mentoring underrepresented science students to **http://www.lssa.ucla.edu/jobs/editjobs.php?id=41** and have three letters of recommendation sent by **June 1, 2006** to: **MARC Director Search, C/O Grace Angus, UCLA LSSA, 621 Charles E. Young Drive South, Box 951606, Los Angeles, CA 90095-1606**. Please use the following job number: **0940-0506-01** in all correspondence.

*UCLA is an Equal Opportunity Employer committed to excellence through diversity.*

**U.S. Environmental Protection Agency**
**Office of Research and Development**
**Research Triangle Park, NC**

# CAREER OPPORTUNITIES IN BIOINFORMATICS AND SYSTEMS BIOLOGY

EPA's Office of Research and Development (ORD) is seeking internationally recognized scientists to fill three positions in the National Health and Environmental Effects Research Laboratory (NHEERL) **http://www.epa.gov/nheerl/** and the National Center for Computational Toxicology (NCCT) **http://www.epa.gov/comptox/**. All positions are located in Research Triangle Park, NC.

ORD plans to fill these positions using EPA's Title 42 Authority, which offers 5-year renewable term appointments at highly competitive, market-level salaries. The positions are part of a larger EPA effort to use advanced technologies in its mission of protecting human health and the environment. Individuals selected for these positions will provide leadership across ORD in their respective areas to conduct a research program that is integrated with activities of the various ORD Laboratories and Centers, and serve as spokesperson for that program. The ideal candidates will have a doctoral level degree in a pertinent science or engineering discipline and 5 or more years of specialized experience. For more information on these positions, please refer to the official job announcements at **http://www.usajobs.opm.gov/**.

**The positions and major duties include:**

### Computational Systems Biologist – NCCT-06-42-01

- Use computational, systems-based models that improve assessment of the public and ecological health implications of environmental stressors.
- Interact with one or more core biological/toxicological disciplines to facilitate the use of this type of knowledge into priority risk assessments.
- Explore how high content "omic" data can be used to understand normal biological processes and link perturbations with adverse health outcomes.

### Systems Biologist – NHEERL-06-42-02

- Develop, or further advance, a critical capability in the area of systems-based models that support improved assessment of the public health implications of environmental stressors.
- Seek opportunities to help integration with the research agendas of other research organizations with similar interests and to define collaborative opportunities.
- Identify emerging issues that would require the use of systems-based approaches for identifying potential solutions.

### Bioinformatician – NCCT-06-42-03

- Conduct independent research and develop new statistical and bioinformatics methods to support the integration of genomic information (broadly defined) in risk assessment.
- Provide lead technical oversight of the extramural STAR Centers for Environmental Bioinformatics and guidance for interactions with intramural scientists.
- Conduct data analysis and develop computer programs for data mining and provide training for EPA researchers in the use of bioinformatics tools.

**Salary and Benefits:** Salary is up to $200,000 per annum, dependent upon qualifications, experience, seniority, and other factors. The selected applicant will be eligible for full benefits including health and life insurance, retirement, and vacation and sick leave.

**How to Apply:** Send a Curriculum Vitae and bibliography with a cover letter that provides your citizenship status, the names of three references, compensation requirements, and a list of the type and amount of resources anticipated to perform the job (e.g., staff, equipment, supplies, software, space). Candidates should reference the specific position number(s) listed above.

Applications should be mailed to the attention of: **Ms. Dorothy Carr, U.S. EPA, MD-C639-02, RTP, NC 27711** or sent via email to **title42@epa.gov** by **May 31, 2006**. For additional information, **Ms. Carr** can also be reached at **(800) 433-9633**.

*The U.S. EPA is an Equal Opportunity Employer.*

## DIRECTOR of NASA Astrobiology Institute (NAI)

The National Aeronautics and Space Administration (NASA) is seeking a Director for the NASA Astrobiology Institute (NAI), which is managed by Ames Research Center in the Silicon Valley, California. Astrobiology is the study of life in the universe, a cross-disciplinary field addressing fundamental questions concerning the origin, distribution, and future of life in the universe. Astrobiology is fundamental to NASA's, and the nation's, vision for Space Exploration. The NAI represents a partnership between NASA, academia and research organizations for the purpose of promoting, conducting, and leading integrated multidisciplinary astrobiology research and for training future researchers. The Institute will showcase modern communications and information technology to bind together institutions and research teams in geographically separated locales to enable an unprecedented degree of remote interaction in pursuit of astrobiology research. Additional information on astrobiology and the Institute can be found at **http://astrobiology.arc.nasa.gov/**.

The NAI Director will identify research opportunities by participating in collaborative research activities with Institute scientists, coordinating efforts across the Institute and in the wider community, and communicating the excitement and achievements of astrobiology and the Institute. The Director should have a strong aptitude for developing revolutionary ways to foster effective collaborations among geographically dispersed scientists.

Applicants must have a strong record of original research in one or more of the astrobiology disciplines; senior-level experience and leadership roles in academic or government administration; effective collaborative partnerships with industry, academia, and other public entities; and a demonstrated commitment to innovative approaches to astrobiology research and training.

The position will be filled through appointment to the Federal Civil Service or through assignment under the Intergovernmental Personnel Act (IPA) by October 1, 2006.

For civil service appointment, all qualified U. S. citizens and current Federal civil servants must submit their applications to Vacancy **#AR06B0054** at the USAJobs website at **http://www.usajobs.opm.gov/** by the vacancy closing date of **May 31, 2006**.

Career employees of a state academic system or certain non-profit organizations may be eligible for IPA assignment. Information on IPA can be found at **http://www.opm.gov/programs/ipa/**. Submit resume describing astrobiology-related leadership, research, and collaborative partnership experience, curriculum vitae and 3 references to: **Ms. Thomasa Nguyen, M/S-241-6, Moffett Field, CA 94035**. Applications must be received by **May 31, 2006**. Contact **Ms. Nguyen** for information about IPA program at **(650) 604-3794**.

*NASA is an Equal Opportunity Employer.*

## CGIAR

### Chair of CGIAR Science Council

### Call for Nominations/Applications

The Consultative Group on International Agricultural Research (CGIAR) (**http://www.cgiar.org/**) is seeking an eminent biological, physical, and social scientists and development expert, including economist, to serve as Chair of its Science Council (SC) (**http://www.sciencecouncil.cgiar.org**).

The CGIAR is the largest publicly funded international agricultural research consortium serving developing countries, has an annual budget of about $ 450 million, and employs over 8000 staff, including more than 1000 scientists, working in over 100 countries.

The SC Chair is selected on the basis of scientific expertise and experience. He/She is appointed on a less than full-time basis (but not less than half time) for an initial period of three years, extendable for up to a total of five years. The SC Chair could function from his or her permanent home base, but would be expected to spend some amount of time in the SC Secretariat in Rome, and would also be expected to maintain close contact with the CGIAR Chair, Director, and Secretariat in Washington, DC. Attractive internationally competitive remuneration will be offered.

Nominations/applications may be submitted on or before **June 9, 2006** at: **http://www.cgiar.org/cgnominations/index.cfm**.

Questions regarding the nomination and selection process may be sent to: **sciencecouncilsearch@cgiar.org**.

# Featured Employers

Search **ScienceCareers.org** for job postings from these employers. Listings updated three times a week.

Abbott Laboratories
www.abbott.com

Genentech
www.gene.com

Kelly Scientific Resources
www.kellyscientific.com

Novartis Institutes for BioMedical Research
www.nibr.novartis.com

Pfizer Biotechnology, Inc.
www.pfizer.com

Pierce Biotechnology, Inc.
www.piercenet.com

If you would like to be a featured employer, call 202-326-6543.

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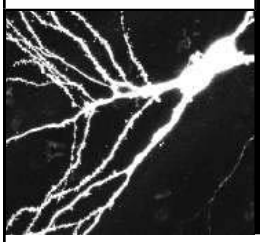

# SENIOR RESEARCH FELLOWSHIPS IN BASIC BIOMEDICAL SCIENCE 2006/2007

UK/Republic of Ireland

**The Wellcome Trust is one of the world's largest biomedical research charities. Our mission is to foster and promote research with the aim of improving human and animal health.**

The purpose of these awards is to provide support in the UK and Republic of Ireland for outstanding young investigators who have shown special promise in their initial studies of basic biomedical problems.

Successful candidates are likely to have a substantial record of publications in their chosen area in leading journals and should be able to demonstrate their ability to carry out independent research.

**For full details please visit the Trust's website at www.wellcome.ac.uk/uksrf**

**Eligibility:** Candidates should normally have between five and ten years' post doctoral research experience. Due allowance will be given to those whose career has been affected either by a late start or by interruption for personal reasons. An individual who holds an established post is not eligible to apply for a Senior Research Fellowship in Basic Biomedical Science to be held at his or her current employing institution.

**Research Support Available:** Fellowships are tenable for up to five years in the first instance and are potentially renewable. The basic salary offered will be determined by the host institution with a fellowship supplement provided by the Trust for the term of the award. The essential costs of the research programme (for example research and technical assistance, consumables and equipment) will also be provided.

**Application Procedure**: A preliminary application form will be available from the Trust's website at www.wellcome.ac.uk/uksrf from 24 April 2006 and completed forms must be returned to the Trust by 9 June 2006.

**Full applications will be invited from 7 July 2006 and should be returned no later than 1 September 2006. Interviews, if invited, will be held in March 2007.**

**Late applications at any stage will not be accepted.**

**NB. Candidates may not apply for more than one Wellcome Trust Fellowship scheme at any one time.**

THE WELLCOME TRUST IS A REGISTERED CHARITY, NO. 210183



# LABORATORY INVESTIGATOR

## EMORY UNIVERSITY SCHOOL OF MEDICINE AFLAC CANCER CENTER & BLOOD DISORDERS SERVICE

The Aflac Cancer Center and Blood Disorders Service at the Emory University Department of Pediatrics and Children's Healthcare of Atlanta is seeking an outstanding laboratory based investigator at the level of Assistant or Associate Professor to join an expanding research program in the areas of vascular biology, adhesion/inflammation or intracellular signaling in the context of sickle cell disease and other hematologic disorders. The successful candidate will join a Division with strengths in stem cell transplantation, oxidant stress, angiogenesis and targeted therapeutics and will be expected to establish an independent research program. The Aflac Cancer Center and Blood Disorders Service is an integral part of the Emory research community and provides a supportive, multidisciplinary environment for developing novel translational therapies for the treatment of sickle cell disease and other non-malignant hematologic disorders. PhD or MD/PhD required. Please direct inquiries with a CV to:

**David Archer PhD**
**Aflac Cancer Center and Blood Disorders Service 2015 Uppergate Drive, Room 422**
**Atlanta, GA 30322**
**darcher@emory.edu or (404) 727-1378**

Aflac Cancer Center & Blood Disorders Service Children's

EMORY UNIVERSITY SCHOOL OF MEDICINE

*Emory University is an EEO/AA employer.*

## Assistant Professor Position

**Department Environmental and Occupational Health**

**Graduate School Public Health**
**University of Pittsburgh**

The University of Pittsburgh is holding an open search for a non-tenure faculty member in the area of environmental Lung genomics. The faculty position is at the Assistant Professor level. Successful candidates will have previous research experience in mechanisms of lung injury and repair in response to environmental and occupational exposure. The candidate should also demonstrate a strong interest to help translate these discoveries to preventive public health strategies. New faculty would be expected to interact with the established groups within the Department of Environmental and Occupational Health, Graduate School of Public Health as well as the Division of Pulmonary Medicine at the School of Medicine. The candidate is expected to contribute to an established research program, and have the ability to develop interdisciplinary research projects. We seek a candidate who can contribute as we move our lung environmental program into greater prominence in ongoing and forthcoming funding opportunities at national and international levels. In addition, participation in teaching of environmental genomics at PhD, MS/MPH levels is also required.

Applications will be received until position is filled. Interested applicants should send letter of intent, a detailed curriculum vitae, a 1-2 page description of research and teaching objectives, and contact information for three references:

**Chair, Search Committee on Environmental Lung Genomics**
**Department of Environmental and Occupational Health**
**Bridgeside Point**
**100 Technology Drive, Rm 328**
**Pittsburgh PA 15219**
**recruitment@eoh.pitt.edu**

*The University of Pittsburgh is an Affirmative Action, Equal Opportunity Employer.*

## U.S. DEPARTMENT OF ENERGY
## Office of Science
## Office of Biological and Environmental Research
## Intergovernmental Personnel Act (IPA)
## Appointment for two Research Program Managers

The U.S. Department of Energy's (DOE) Office of Biological and Environmental Research (OBER), Office of Science, is soliciting applications from university scientists interested in a 2-year assignment (with an option for an additional 2 years) under an Intergovernmental Personnel Act (IPA) assignment. The respective incumbents will serve as research program managers in the Climate Change Research Division of OBER located in Germantown, Maryland. One assignee would serve as the research program manager for the Terrestrial Carbon Sequestration Research Program and manage selected components of DOE's Terrestrial Carbon Cycle Research Program. Information on these two programs is available at: **http://cdiac2.esd.ornl.gov/index.html** and **http://www.science.doe.gov/ober/CCRD/tcp.html**, respectively. A second IPA assignee would manage DOE's Integrated Assessment Research Program. Information on the Integrated Assessment Research Program is available at: **http://www.science.doe.gov/ober/CCRD/ia.html**. To ensure that research sponsored by these programs is effectively coordinated within and among these and other Climate Change Research Programs in DOE, the incumbents would be expected to work in a team setting with other program managers in OBER.

An IPA assignment is a temporary transfer of skilled personnel between the Federal Government and State or local governments, institutions of higher education, Native American tribal governments, and eligible non-Federal "other organizations," including Federally Funded Research and Development Centers. Assignments are implemented through written Assignment Agreements between DOE, the non-Federal employer, and the assignee. For information on Intergovernmental Personnel Act assignments, please refer to the DOE Directive on IPAs at **http://www.directives.doe.gov/cgi-bin/explhcgi?qry1347894433;doe-110**.

Individuals interested in either IPA assignment position are requested to send their resume to: **Dr. Jerry W. Elwood, Director, Climate Change Research Division, Department of Energy, SC-23.3/Germantown Building, 1000 Independence Avenue, SW, Washington, DC 20585-1290 (Phone: 301-903-3281, email: (jerry.elwood@science.doe.gov)**. Applicants are requested to describe their expertise and experience that would enable them to effectively manage the Terrestrial Carbon Sequestration Research and Carbon Cycle Research Program or manage the Integrated Assessment Research Program. These vacancies will remain open until filled. Prior to submitting an application, prospective applicants should know whether their employer would approve the temporary assignment through the IPA Assignment Agreement.

www.cam.ac.uk/jobs/

## The Goldsmiths' Professorship of Materials Science

The Board of Electors to the Goldsmiths' Professorship of Materials Science (the senior chair in the Department of Materials Science and Metallurgy) invite applications for this Professorship from persons whose work falls within the field of Materials Science, interpreted in the broadest possible terms.

The Professorship falls vacant on 1 October 2008 but, to aid succession planning, the University hopes to be in a position to make an appointment from 1 October 2006 or as soon as possible thereafter. The successful candidate will hold a fixed-term Professorship on appointment until taking up the Goldsmiths' Professorship of Materials Science on 1 October 2008.

**Informal enquiries may be made to Professor Lindsay Greer, Head of the Department of Materials Science and Metallurgy, tel (01223) 334308, e-mail: alg13@cam.ac.uk**

## The Woodwardian Professorship of Geology

The Board of Electors to the Woodwardian Professorship of Geology invite applications for this Professorship from persons whose work falls within the field of Earth Sciences.

The Professorship falls vacant on 1 October 2008 but, to aid succession planning, the University hopes to be in a position to make an appointment from 1 October 2006 or as soon as possible thereafter. The successful candidate will hold a fixed-term Professorship on appointment until taking up the Woodwardian Professorship of Geology on 1 October 2008.

**Informal enquiries may be made to Professor E Salje, Head of the Department of Earth Sciences tel: (01223) 333481, e-mail: es10002@esc.cam.ac.uk**

**Further information for both the above posts may be obtained from the Academic Secretary, University Offices, The Old Schools, Cambridge CB2 1TT, (e-mail: ibise@admin.cam.ac.uk), to whom a letter of application should be sent, together with details of current and future research plans, a curriculum vitae, a publications list and form PD18 with details of two referees, so as to reach him no later than 9 June 2006.**

**Please indicate which position you are applying for.**

UNIVERSITY OF CAMBRIDGE

The University offers a range of benefits including attractive pension schemes, professional development, family friendly policies, health and welfare provision, and staff discounts. The University is committed to equality of opportunity.

STANFORD UNIVERSITY

## EXECUTIVE DIRECTOR
## Global Climate and Energy Project

The Global Climate and Energy Project (GCEP) solicits applications for the position of Executive Director. GCEP is a ten-year, $225 million project devoted to fundamental science and engineering science research leading to new options for reducing greenhouse gas emissions associated with energy use. We seek a person of high stature in energy research with a demonstrated record of research in an energy area related to GCEP's research activities. The ideal candidate will be a research scientist or engineer who also has significant experience in developing and managing a research portfolio. We anticipate that this appointment will be made as a research faculty or senior fellow associated with a department or institute appropriate to the background of the successful candidate. Funding for this position will be provided by GCEP. The successful applicant will interact effectively with a broad range of Stanford colleagues, including physical, chemical, and biological scientists, and engineers, as well as representatives of the project sponsors. The search is open to applicants at the level of full professor or senior fellow. The position will remain open until filled.

Applications, including a curriculum vita, a statement outlining research interests and research management approach, and the names and addresses of three or more referees, should be sent by **May 15, 2006**, via either electronic or regular mail, to:

**GCEP Executive Director Search Committee, 416 Escondido Mall Stanford University Stanford, CA 94305-2205 gcep_search@stanford.edu**

Questions can be directed to **Prof. Franklin M. Orr, Jr.** at: **gcep_inquiries@stanford.edu**

Information about GCEP can be found at: **http://gcep.stanford.edu**

*Stanford University has a strong institutional commitment to the principle of diversity. In that spirit, we particularly encourage applications from women, members of ethnic minorities, and individuals with disabilities.*

# MICHIGAN STATE UNIVERSITY

## Assistant Professor - Postharvest Biologist

The Department of Horticulture at Michigan State University invites applications for 9-month tenure-track Assistant Professor position in Postharvest Biology. The successful candidate will have teaching (25%) and research (75%) responsibilities consistent with the missions of the Agricultural Experiment Station, College of Agriculture and Natural Resources, and Michigan State University. We seek a highly qualified individual with excellent communication skills who will develop an externally-funded, nationally competitive program using innovative technologies to answer fundamental questions related to postharvest biology. It is expected that this individual will establish interactions across disciplinary lines, and will address questions of relevance to harvested plant products and long-term goals of associated plant industries. Possible research directions include mechanisms of ripening or senescence, factors influencing phytoceutical content and texture/flavor/aroma/color development of technologies to reduce loss of key quality attributes, and amelioration of abiotic or biotic factors leading to postharvest losses. The Department of Horticulture possesses excellent facilities dedicated to postharvest studies including an eight-laboratory suite, 23 walk-in controlled temperature rooms, a cold extraction room, two walk-in freezers, and extensive analytical instrumentation and supporting equipment. In addition, excellent opportunities exist for interactions with industry and researchers in our large plant science community and others at MSU. The individual will also be expected to develop courses in post-harvest biology as determined by the needs of the department.

Applicants must possess a Ph.D. in a relevant area with evidence of research productivity. Postdoctoral and/or industry experience, teaching experience, evidence of scholarly activities and ability to obtain competitive external grants in support of research and/or educational programs are desirable. Screening will begin September 1, 2006 and will continue until a suitable applicant is identified. The expected starting date is January 1, 2007. Submit a letter of application, curriculum vitae, statement of research and teaching relevant to this position, and three reference letters to: **Prof. Muralee G. Nair, Search Committee Chair, 420 Plant and Soil Science Building, Department of Horticulture, Michigan State University, East Lansing, MI 48824-1325**.

MSU IS AN AFFIRMATIVE ACTION, EQUAL OPPORTUNITY INSTITUTION.

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# dkfz. DEUTSCHES KREBSFORSCHUNGSZENTRUM IN DER HELMHOLTZ-GEMEINSCHAFT

As the leading biomedical research centre in Germany our programs focus on basic and translational cancer research.

We have an immediate opening for the position of a

## Director of Light Microscopy Core Facility

(ref. 57/2006)

at the DKFZ.

In the facility, Zeiss LSM microscope systems are used with both living and fixed biological specimens from a variety of sources, ranging from yeast to tissue sections. These are further analyzed with several computer image manipulation packages, including MetaMorph and Zeiss Image Examiner. In addition, an Electron Microscopy Facility may be incorporated in the future.

Candidates are supposed to hold a PhD and be experienced in various microscopy and image analysis techniques.

The Facility Director will work with a Microscopy Assistant to maintain and upgrade appliances and to train and supervise users. The Director will also be involved in exploring new fields and technologies (new equipment and technology purchases) and will have many opportunities for collaboration on users' research projects.

DKFZ wish to increase the proportion of female scientists and strongly encourage applications of qualified women. With equal qualifications, handicapped individuals will be considered preferentially.

Applications with a full CV, a brief concept for the core facility, and two names of potential referees shall be sent within 3 weeks after this announcement to:

DKFZ
Personal- und Sozialwesen
Im Neuenheimer Feld 280
D-69120 Heidelberg
www.dkfz.de

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# University of Zurich

In an effort to further strengthen structural biology within the Life Science Zurich programs, the University of Zurich is seeking to fill a position of a

## Professor of Structural Bioinformatics

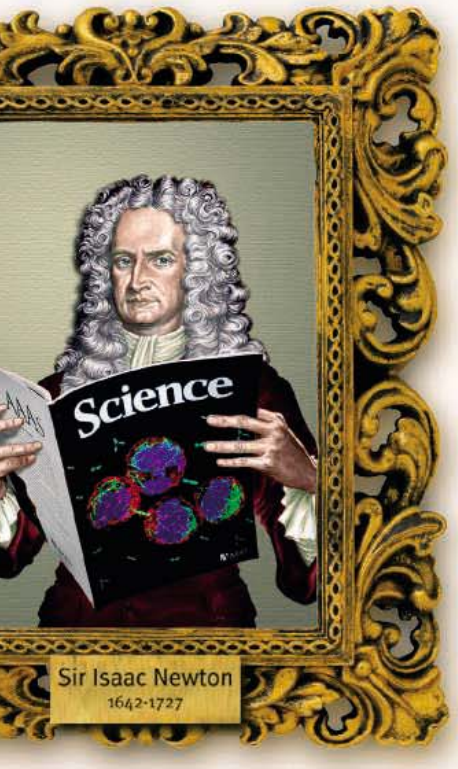
The appointment (Department of Biochemistry) associated with the Faculty of Science and the Faculty of Medicine will be made at the assistant professor level (non-tenure track).

We are searching for individuals with an outstanding potential in the development and application of new bioinformatics techniques and algorithms. Candidates should have a background in life sciences and experience in the analysis of genomic, proteomic and particularly structural data sets. Research interests in the area of protein-protein interactions, fold/structure prediction and homology modeling would be welcome. Outstanding individuals with an experimental structural biology research program and strong in the bioinformatics application will also be considered.

The Institute of Biochemistry is located on the Irchel campus, where most of the biological and physical science Institutes of the University are also situated. Nearby are the Science Departments of the ETH Zurich, and the Paul-Scherrer-Institut, which houses the newly established synchrotron Swiss-Light Source for structural studies of biomolecules. The density of biomolecular science in Zurich provides a highly stimulating and very attractive environment for interdisciplinary research. In addition, strong ties in teaching and research are being established between corresponding units of the University and the ETH Zurich. In particular, a National Center of Competence in Structural Biology (**http://www.structuralbiology.unizh.ch**) was established in Zurich to integrate and expand structural biology and related areas.

The new professor will be expected to contribute to teaching. Applications including a curriculum vitae, list of publications and an outline of current and future research plans should be received not later than June 16, 2006. Please send your application to the Vice-Dean of the Faculty of Science, Professor Daniel Wyler, Dekanat der Mathematisch-naturwissenschaftlichen Fakultät der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland. The CV, list of publications and the research outline should also be submitted as a single file (PDF) to **jobs@mnf.unizh.ch.** For additional information see also **http://www.unizh.ch** or contact Prof. Markus Grütter (**gruetter@bioc.unizh.ch**), Universität Zürich, Winterthurerstrasse 190, CH-8057 Zürich.

The University of Zurich is an equal opportunity employer. Applications from women candidates are particularly encouraged.




# Looking for a great science career?

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Your career is too important to leave to chance. So to find the right job or get career advice, turn to the experts. At ScienceCareers.org we know science. And we are committed to helping take your career forward. Our knowledge is firmly founded on the expertise of *Science*, the premier scientific journal, and the long experience of AAAS in advancing science around the world. Put yourself in the picture with the experts in science. Visit www.ScienceCareers.org.

## POSITIONS OPEN

### POSTDOCTORAL FELLOWSHIP
**Epithelial Cell Biology and Signaling in Kidney Disease**

NIH Training Grant positions are available immediately in the Renal Division at Brigham and Women's Hospital, laboratory of **Dr. Bradley M. Denker.** *Applicant must be a U.S. citizen or permanent resident.* Research is focused on G protein signaling in epithelia to study (1) tight junctions, (2) signaling in Polycystic Kidney Disease and (3) glomerular epithelial cells. Ample opportunities will be provided for the independent design and implementation of experiments, in close consultation with the Principal Investigator and other members of a collegial and dynamic research group.

Skills required: The successful applicant will be a highly motivated scientist able to work well within a group, think independently, and to frame experimental strategies in broad scientific terms, rather than as technical exercises. A strong background in molecular biology, biochemistry, and imaging is helpful, as is a desire to learn and apply emerging methods.

Education and experience: Applicants must have a Ph.D. and/or M.D. with less than two years of postgraduate experience.

Send curriculum vitae to:

**Bradley M. Denker, M.D.**
**Renal Division, Brigham and Women's Hospital**
**Harvard Institutes of Medicine**
**77 Avenue Louis Pasteur**
**Boston, MA 02115**
**E-mail: bdenker@rics.bwh.harvard.edu**

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### DEPARTMENT CHAIR

The Department of Chemistry and Biochemistry at the University of Maryland, Baltimore County (UMBC) invites applications and nominations for the position of Department Chair. The Chair will be expected to provide vigorous leadership for a growing department of eighteen tenured or tenure-track faculty and four instructors. The Department offers Ph.D. degrees in both chemistry and biochemistry, and applications are invited from internationally recognized scholars in any area of these disciplines who have a commitment to research and quality teaching at both the undergraduate and graduate levels.

UMBC, a Carnegie ranked research university, is a member of the University System of Maryland and is located in a Baltimore suburb about 35 miles from Washington, D.C. To apply send a resume and the names of three persons who can be contacted for supporting letters to: **Dr. M.F. Summers, Department of Chemistry and Biochemistry, 1000 Hilltop Circle, Baltimore, MD 21250.** Applications will be accepted until the position is filled. *UMBC is an Equal Opportunity/Affirmative Action Employer. Minority, women, and individuals with disabilities are encouraged to apply.*

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The Ecological Genomics Visiting Scholar Program provides **SABBATICAL OPPORTUNITIES** at Kansas State University for permanent faculty researchers interested in ecological genomics. Our multi-disciplinary Institute seeks to understand responses of organisms to their natural environment by combining functional genomic and ecological approaches. Applicants should contact the **Principal Investigator** of a potential host laboratory at Kansas State University **(website: http://www.ksu.edu/ecogen/resgroup.html)** to explore space availability and overlap in research interests. Excellent University resources include the Konza Prairie Long Term Ecological Research (LTER) site, Division of Biology infrastructure, and gene analysis facilities. Scholars will receive a generous stipend and monetary research supplement. Application instructions, information about the Ecological Genomics Program and our Annual Symposium, November 3 through 5, 2006, are at **website: http://www.ksu.edu/ecogen.** Review of applications will begin July 1, 2006. This ad is paid for by Kansas State University (KSU). *KSU is an Equal Opportunity/Affirmative Action Employer, and actively seeks diversity among its employees.*

## POSITIONS OPEN

### COMPUTATIONAL BIOLOGIST
**Tenure Track Faculty Position**

The Department of Computer Science at the University of New Orleans (UNO) and the Research Institute for Children seek an individual who combines strong computational skills with laboratory expertise, and who is addressing a problem of biomedical importance. The appointment will be at the rank of **ASSISTANT PROFESSOR,** although higher rank may be considered. Very competitive salary, startup funds, and ongoing support are offered. Qualifications include a Ph.D. or postdoctoral experience in computer science and strong practical skills in the biological laboratory. Responsibilities include classroom teaching, supervision of graduate students, securing external funding, and publication of research results.

UNO is an urban public university, with $40 million a year in sponsored research funding. The Computer Science Department has over 300 undergraduate, 60 Master's and 11 doctoral students, and state-of-the-art computational facilities. The Research Institute for Children is an independent institute within the Louisiana State University system, housing faculty from both UNO and LSU Health Sciences Center. Areas of active research include host-pathogen interactions, immunology, and diabetes.

Applicants should respond by e-mail, sending a resume and a statement of research interests, along with names of references to **e-mails: search@cs.uno.edu** and **spincus@chnola-research.org.**

*UNO and the Research Institute for Children are Equal Opportunity Employers.*

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### CURATOR OF HERPETOLOGY

The Wildlife Conservation Society (WCS) is seeking an individual with a Ph.D. in herpetology, or closely related field, for the position of Curator of Herpetology at the Bronx Zoo. The qualified candidate will be responsible for Departmental operations and herpetological exhibits at the Bronx Zoo, and will assume the role of chief advisor for similar exhibits at all WCS facilities. The candidate will also be encouraged to work with WCS field herpetologists and participate in field conservation programs. It is expected that the Curator of Herpetology will become a leader of and strong advocate for upcoming major WCS program to address the current amphibian crisis. Candidates for the position must have published in peer-reviewed journals, and be currently involved in conservation research as well as a variety of herpetological endeavors and associations. The qualified candidate will be expected to carry on the Herpetology Department's long-standing program of excellence in animal husbandry and conservation research, as well as create a vision for the future of the Department and of herpetology at the Bronx Zoo.

Benefits include generous vacation, medical/dental, 401K, pension, and onsite parking. Salary commensurate with experience. E-mail resume/cover letter/salary requirement to **e-mail: hr@wcs.org.** Type LKREPT in subject.

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### NATIONAL UNIVERSITY OF SINGAPORE
**Department of Chemical and Biomolecular Engineering**

The Department of Chemical and Biomolecular Engineering at National University of Singapore invites applications for **TENURE-TRACK FACULTY** positions at all levels. The Department is one of the largest internationally with excellent in-house infrastructure for experimental and computational research. A Ph.D. in chemical engineering or related areas and a strong research record with excellent publications are required. Please refer to **website: http://www.chbe.nus.edu.sg/** for more information on the areas of interest and for application details. Applicants should send full curriculum vitae (including key publications), a detailed research plan, a statement of teaching interest, and a list of names of at least three references to: **Professor Raj Rajagopalan, Head of Department (Attention: Ms. Nancy Chia, e-mail: nancychia@nus.edu.sg).**

## POSITIONS OPEN

### ASSISTANT / ASSOCIATE / FULL PROFESSOR
**Cancer Biology or Drug Therapy**

The Department of Pharmaceutical Sciences, Texas Tech University Health Sciences Center (TTUHSC), seeks applicants for an attractive new tenure-track faculty position at the **ASSISTANT/ASSOCIATE/FULL PROFESSOR** level. The successful candidate will join a vibrant and expanding group of extramurally funded biomedical and pharmaceutical cancer researchers **(website: http://www.ttuhsc.edu/sop/pharmsci)** who are part of a newly developed university cancer institute. Applicants must have a doctoral degree with research experience in any aspect of cancer biology, chemotherapeutic drug resistance, anticancer drug development or delivery. The successful candidate will be expected to develop an extramurally funded research program as well as teach Pharm.D. and graduate Ph.D. students. Candidates with current NIH funding who have training in pharmacology or drug therapy are particularly encouraged to apply. Competitive salary, start-up package, and laboratory space are available. Applicants should submit documents online at **website: http://jobs.texastech.edu** (job requisition number 61308). Please include curriculum vitae, a summary of research interests, and names and addresses of three references. TTUHSC in Amarillo includes the School of Pharmacy, School of Medicine and the Harrington Cancer Research Center. The Department has 23 full-time faculty with interests in cancer biology, brain/vascular, and pharmaceutical research, and state-of-the-art core facilities. For questions, contact the Search Committee Chair, **Dr. U.S. Rao. E-mail: us.rao@ttuhsc.edu. Telephone: 806-356-4015, extension 294.** *TTUHSC is an Equal Opportunity/Affirmative Action Institution. Minorities and women are encouraged to apply.*

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## EVOLUTIONARY COMPUTATIONAL GENOMICS/BIOINFORMATICS ASSOCIATE/FULL PROFESSOR

### INDIANA UNIVERSITY, BLOOMINGTON

The Department of Biology and the School of Informatics jointly seek an established scholar with a dynamic and innovative research program in the area of bioinformatics and/or computational biology. Successful candidates will be expected to contribute to and complement existing research and teaching strengths in comparative genomics, molecular evolution, and/or population genetics, although other areas of research expertise are not precluded. Indiana University is in a period of significant expansion in the life sciences, and candidates must have a demonstrated ability to promote an environment of collaborative scholarship and a vision for the development of future research and training activities. Candidates with a laboratory component to their research are encouraged to apply.

A complete curriculum vitae, a statement of current and future research interests, and contact information for at least three letters of reference should be forwarded to: **Jeremy Bennett, Department of Biology, Indiana University, Bloomington, IN 47405** (hard mail) or **jebennet@indiana.edu** (pdf files). Direct inquiries about the position should be made to: **Michael Lynch, milynch@indiana.edu**. Review of applications will begin on **June 1, 2006**, and will continue until the position is filled.

*Indiana University is an Affirmative Action/ Equal Opportunity Employer. Women and minority candidates are encouraged to apply.*

## President

IES

### Institute of Ecosystem Studies

The Institute (**www.ecostudies.org**), established in Millbrook, New York, in 1983 by Dr. Gene E. Likens, is dedicated to the creation, dissemination and application of knowledge about ecological systems. With a staff of over 100, the Institute has become one of the world's premium ecological research centers.

As the executive officer, the President is responsible for the leadership of the Institute. The successful candidate must have significant credentials in environmental sciences or related disciplines with a demonstrated record of successful senior management.

Inquiries, applications and nominations should be sent to:

**Malcolm MacKay**
**Managing Director**
**Russell Reynolds Associates, Inc.**
**200 Park Avenue, 23rd floor**
**New York, New York 10166**
**IESpresident@russellreynolds.com**

*The Institute is an Equal Opportunity Employer. Women and minority group members are encouraged to apply.*

## DIRECTOR OF STEM CELL BIOLOGY (TISSUE ENGINEERING)

### *Department of Cell Biology and Molecular Medicine*

The University of Medicine and Dentistry of New Jersey - New Jersey Medical School, Department of Cell Biology and Molecular Medicine, is seeking applicants for tenure or research track faculty position as the Director of Stem Cell Biology (Tissue Engineering). Applicants should have a PhD or MD, and have demonstrated excellence in basic science research, as confirmed by National Research Funding.

Strong core facilities in pathology, physiology and experimental surgery are available to support the cellular/molecular sciences. Tenure track applicants also need excellent English and communication skills.

Curriculum vitae including names of at least three references, and a description of research interests should be sent to: **Stephen F. Vatner, MD, Chair, Department of Cell Biology and Molecular Medicine, Cardiovascular Research Institute, UMDNJ-New Jersey Medical School, 185 South Orange Avenue, MSB G609, PO Box 1709, Newark, NJ 07101-1709.** UMDNJ is an AA/EO Employer, M/F/D/V and is a member of the University Health Systems of New Jersey. For more information, visit **www.umdnj.edu/hrweb.**

UMDNJ
NEW JERSEY MEDICAL SCHOOL
University of Medicine & Dentistry of New Jersey

## POSITIONS OPEN

### ASSISTANT/ASSOCIATE PROFESSOR
**Anatomical Sciences/Neurobiology**
**University of Louisville**

The Department of Anatomical Sciences and Neurobiology is seeking applications for a tenure-track position at the rank of either Assistant or Associate Professor. The successful candidate must have a Ph.D. and/or M.D. degree, an outstanding publication record, and a NIH-funded research program that is focused in neurosciences. Current strengths in the Department include molecular and developmental neurobiology, neural plasticity, or sensory systems. There are excellent opportunities for collaboration. The successful candidate will be expected to contribute to the medical and graduate teaching missions of the Department; interest in teaching histology is desirable. Review of applications will begin immediately. Information about the Department can be found at **website: http://www.louisville.edu/medschool/anatomy.**

Please send curriculum vitae and statement of research interests/plans, along with three letters of reference to: **Dr. Mengsheng Qiu, Anatomical Sciences and Neurobiology Faculty Search Committee, Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Health Sciences Center, Louisville, KY 40292.**

*The University of Louisville is an Affirmative Action/Equal Opportunity Employer. The University of Louisville encourages women and minorities to apply.*

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### POSTDOCTORAL POSITION
**Cellular Neurophysiology of Cortical Information Processing**

A Postdoctoral position is available for a project that examines how output regions of the basal ganglia influence information processing in the neocortex. Our strategy is to integrate in vivo single-unit recordings from rodents performing behavioral tasks with in vitro intracellular recordings from brain regions important for those tasks (e.g. premotor cortex and ventrolateral thalamus). The immediate need is for a scientist interested in participating in the in vitro work, which will take advantage of fluorescent tracers to identify the connectivity of recorded neurons as well as use the dynamic clamp method to simulate naturally occurring patterns of synaptic input.

Candidates should have experience with patch clamp recordings in brain slices and/or anatomical methods to map the connectivity of neuronal circuitry. The project is best suited to individuals with an interest in the function of the neocortex at the cellular and systems levels of analysis.

Submit curriculum vitae, statement of research interests, and names of three references to: **Dr. Niraj Desai, The Neurosciences Institute, 10640 John Jay Hopkins Drive, San Diego, CA 92121. E-mail: jobs@nsi.edu.**

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Applications are invited for a tenure-track position in fish biology at the **ASSISTANT/ASSOCIATE PROFESSOR** level. Position is for nine-month teaching, research, and academic service. Summer salary is dependent upon availability of research grants. Incumbent is expected to teach undergraduate and graduate courses. Ph.D. degree in fish biology, fisheries, or closely related area is required. Preference is for field-oriented scientists with potential to obtain extramural funding and expertise in one or more of the following areas: fish population dynamics or genetics; aquatic habitat assessment, restoration or manipulation; limnology; conservation; or ecophysiology. Applications can be submitted by visiting **website: http://jobs.texastech.edu/hr** (requisition number 61280). Five letters of reference should be sent directly to: **Carlton Britton, Chair, Search Committee, Range, Wildlife, and Fisheries, Texas Tech University, Lubbock, TX 79409-2125, e-mail: carlton.britton@ttu.edu.** Review of applications will begin 1 June 2006, and continue until position is filled. *Texas Tech University is an Equal Employment Opportunity/Affirmative Action Institution.*

## POSITIONS OPEN

### TWO TENURE-TRACK POSITIONS
**Translational Neuroscience**
**Case Western Reserve University**

As part of a new initiative in translational neurosciences between Case Western Reserve University and University Hospitals of Cleveland, the Translational Neuroscience Department invites applications for two tenure-track positions. These positions are designed to be filled at the **ASSISTANT PROFESSOR** level, but exceptionally qualified candidates for other levels will be eligible. All areas of translational neuroscience will be considered, including molecular/genetic, cellular, and preclinical/clinical studies. Candidates in the following areas would best complement emerging strengths in the program: (1) brain tumors biology, including cellular and molecular studies of tumor cell migration, proliferation, and death with an emphasis on animal models and (2) stroke, including neuronal cell death, neovascularization, and neuronal metabolism. The Center is housed in newly renovated space, adjacent to the Department of Neurosciences and provides access to state-of-the-art facilities including confocal and laser capture microscopy, transgenic animal facilities, fluorescence activated cell sorter (FACs), and microarray analyses. Successful applicants will be expected to develop effective interactions with clinical and basic faculty in the neurosciences and neurological surgery as well as in the National Center for Stem Cell and Regenerative Medicine. Generous startup funds are available. Candidates should possess a Ph.D./M.D. degree, and have several years of postdoctoral experience. They will be expected to develop a successful, funded research program and participate in multiperson translational clinical and preclinical programs. Please submit curriculum vitae, a summary of research interests, and the names of three references to: **Dr. Robert H. Miller, Director, Center for Translational Neurosciences, Department of Neurosciences and Neurological Surgery, Case Western Reserve University School of Medicine, 10900 Euclid Avenue, Cleveland, OH 44106-4975.** *Case Western Reserve University is an Equal Opportunity/Affirmative Action Employer.*

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### FACULTY POSITION
**University of Wisconsin, Madison**
**Integrative Physiology**

The Department of Comparative Biosciences, School of Veterinary Medicine invites applications for a tenure-track faculty position, **ASSISTANT OR ASSOCIATE PROFESSOR.** Qualifications include a Ph.D., postdoctoral experience, commitment to excellence in teaching, and the ability to develop an extramurally funded research program in an area of physiology integrating cellular and molecular studies in a whole organism perspective. Teaching responsibilities include participation in a physiology course in the veterinary medical curriculum and/or the undergraduate level. To apply, send curriculum vitae, brief statements of research interests and teaching philosophies, and three letters of reference to: **Gordon S. Mitchell, Chair, Department of Comparative Biosciences, University of Wisconsin, 2015 Linden Drive, Madison, WI 53706.** Apply by July 1, 2006. For additional information, see **website: http://www.vetmed.wisc.edu/jobs.html.** *Equal Opportunity/Affirmative Action Employer.*

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### MEDICAL WRITER

Physicians' Education Resource (PER) is seeking a Medical Writer/Editor to join its team. PER is a medical education company, located in Dallas, Texas, specializing in the field of oncology. Successful candidates will be responsible for writing manuscripts from original data, reporting highlights from cancer meetings, creating slide sets for pharmaceutical companies, and editing and rewriting author-submitted manuscripts. This full-time position requires a Ph.D. in a biomedical science. Send resume and salary requirements to: **Barb Schmaedeke, Human Resources Director, 3535 Worth Street #185, Dallas, TX 75246. E-mail: hr@perlp.com.**

## POSITIONS OPEN

### BIOCHEMIST
**University of Victoria, Victoria, British Columbia, Canada**

The Department of Biochemistry and Microbiology invites applications for a tenure-track **ASSISTANT PROFESSOR** position in biochemistry. The Department has particular research expertise in macromolecular structure/function, cell signaling, genome dynamics, and infectious disease. Applications from excellent scientists in these and other areas of biochemistry will be considered. The University of Victoria offers outstanding research infrastructure, including a proteomics platform, DNA sequencing center, and X-ray crystallography facilities. There are ample opportunities to collaborate with researchers in the Faculty of Science, the Island Medical Program, and the British Columbia Cancer Research Centre. The appointee will be expected to develop a rigorous, independent research program, funded by external support, and participate in teaching the undergraduate biochemistry curriculum. The start date may be as early as January 2007.

Qualifications include a Ph.D., postdoctoral experience, demonstrated research excellence, and teaching potential. Letters of application should clearly outline research interests, and should be accompanied by curriculum vitae, and the names and contact information of at least three referees. Consideration of applications will start July 1, 2006.

All qualified candidates are encouraged to apply; however, in accordance with Canadian Immigration requirements, *Canadians and permanent residents will be given priority.* Contact:

**Dr. C.G. Cupples, Chair**
**Department of Biochemistry and Microbiology**
**University of Victoria**
**P.O. Box 3055 STN CSC**
**Victoria BC V8W 3P6**
**Canada**
**E-mail: biocmicr@uvic.ca**
**Website: http://web.uvic.ca/biochem**

*The University of Victoria is an equity employer and encourages applications from women, persons with disabilities, visible minorities, Aboriginal Peoples, people of all sexual orientations and genders, and others who may contribute to the further diversification of the University.*

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### FACULTY POSITIONS
**Lung Pathobiology**

The Ohio State University, Division of Pulmonary, Allergy, Critical Care and Sleep Medicine **(website: http://www.internalmedicine.osu.edu/pulmonary/index.cfm)** and the Dorothy Davis Heart and Lung Research Institute **(website: http://heartlung.osu.edu/)** are seeking four basic science faculty working in areas related to human genetics of lung disease, acute lung injury/sepsis, or chronic inflammation and repair of the lung. Ph.D. scientists or physician-scientist candidates with a strong record of publications and grant support are encouraged to apply. Send curriculum vitae, statement of research interests and direction, three reference contacts, and cover letter to: **Dr. Clay Marsh, Division Director, The Ohio State University, Pulmonary Critical Care, Allergy and Sleep Medicine, 201 HLRI, 473 W. 12th Avenue, Columbus, OH 43210. Telephone: 614-247-7707; e-mail: clay.marsh@osumc.edu.** *The Ohio State University is an Equal Opportunity/Affirmative Action Employer. Qualified women, minorities, Vietnam era veterans, and individuals with disabilities are encouraged to apply.*

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# DUAL MASTERS IN "BRAIN AND MIND SCIENCES"

**Université Pierre et Marie Curie in partnership with the Ecole Normale Superieure and University College London**

This new 2-year international Masters level programme in brain and mind sciences is offered by three of Europe's most prestigious centres of research and teaching in cognitive studies and neuroscience.

Applications for places for the 2006-2008 session are invited from outstanding students with applications from countries outside the European Union equally welcomed.

The programme will include a year spent in LONDON and a year in PARIS. Students will graduate with a Masters from UCL, a Masters level university diploma from UPMC/ENS and a DUAL MASTERS DEGREE IN BRAIN AND MIND SCIENCES awarded by the three institutions in partnership.

Students will be rigorously selected on the basis of academic excellence and academic recommendation. A maximum of 20 students will be accepted per academic year, 10 starting in PARIS and 10 in LONDON with cross-over after a year. Fees will be payable and scholarships may be available in cases of material need.

The programme is designed to give students a personalized cursus of study through lectures and research projects in neuroscience and cognitive science disciplines relevant to the Brain and Mind Sciences. Students will be able to re-orient; to apply different disciplines/competencies already acquired in pre-Masters study (eg engineering, mathematics, genetics); to study basic and clinical neuroscience or cognitive science topics in depth or broadly.

The course is designed to cater for students' individual interests and needs by access to major themes through existing established Masters programmes. Depending on choice of modules students can aim to obtain:
(1) A theoretical grounding in neurobiological and cognitive research including philosophy of science, methods (including imaging, psychophysics and neuropsychology), molecular, cellular, genetic and integrative neuroscience.
(2) An appreciation of the way Brain & Mind questions can be approached theoretically and experimentally in humans and other model systems.
(3) An appreciation of the interaction between theory, modelling and empiricism in tackling Brain & Mind problems
(4) Practical experience of investigating Brain & Mind problems from two cultural perspectives (in the two cities).

Students should be prepared to follow lectures in English or French. Language classes will be available in both cities. Examinations and dissertations may be written in English in both cities.

For further information consult **http://www.ion.ucl.ac.uk/education/msc-brain-mind.htm** Applications in either English or French should be sent to either Dr Caroline Selai, Institute of Neurology, Queen Square, London WC1N 3BG, UK (email **c.selai@ion.ucl.ac.uk**) or Ann Lohof, Laboratoire DVSN, UMR 7102, Case 14, Université P et M Curie, 9 quai St Bernard, 75005 Paris (email **Ann.Lohof@mail.snv.jussieu.fr**), in the form of a curriculum vitae with achieved or expected examination scores, a personal statement/letter of motivation of not more than one A4 page, the names and email addresses of two academic referees and a letter of recommendation with confirmation of predicted grades from the student's Dean of Faculty or University President.

## POSITIONS OPEN

### ASSOCIATE RESEARCH SCIENTIST CT/MICRO-CT LABORATORY
**University of Iowa, Department of Radiology**

To provide scientific and technical leadership in the management, design, and conduct of CT/micro-CT research and development with an emphasis on biomedical applications associated with projects either funded or for funding. A Ph.D. or equivalent professional degree is required with specific areas of study relating to mathematics, computer science, image analysis and visualization, with demonstrated knowledge of state-of-the-art CT/Micro-CT imaging. A history of progressively responsible research work should be evident. The applicant must have an established track record of publications in the tomographic imaging field. Desirable qualifications include experience in programming with C/C++/Matlab and experience in image processing/analysis and visualization. Prior experience successfully securing external grant funding is desirable. To apply for this position number 52571, visit our **website: http://jobs.uiowa.edu.** Initial screening of applications begins immediately. *Women and members of minority groups are encouraged to apply. The University of Iowa is an Affirmative Action/Equal Opportunity Employer.*

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### CHEMISTRY MANUFACTURING SUPERVISOR, SUNNYVALE, CALIFORNIA

Responsible for the smooth production of all chemistry related products. Knowledge of PCR techniques and methodology are essential, and experience in multiplex PCR assays desirable. Requires a B.S. in life sciences with over five years of relevant professional experience, M.S. in life sciences with over two years of relevant professional experience preferred. Must have hands-on working knowledge of laboratory instrumentation (spectrophotometers, fluorometers, thermocyclers) and associated application software. Experience in a regulated manufacturing environment (ISO or good manufacturing practice) a must. Proficient in Microsoft Office (Word, Excel, PowerPoint), data handling, data analysis, and presentation. To be considered, please submit your resume online at **website: http://www.cepheid.com.** You may also e-mail your resume to **e-mail: talent@cepheid.com** or fax it to **fax: 408-541-4193, Attn: Talent Acquisition Group.** (Job code RB293MN)

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### CHIEF MEDICAL OFFICER

Highly intelligent individual with exceptional communication skills sought by prominent Manhattan family to research and coordinate family medical and healthcare issues. Act as liaison with leading medical researchers and consultants in academia and industry, with full responsibility for technical, financial, and administrative functions. Considerable weight given to evidence of unusual academic or other intellectual distinction. Ph.D. or M.D. required, clinical experience a plus but not essential. Possible entrepreneurial opportunities involving delivery of ultrahigh-end medical care to other, similar families. Full- time position. Excellent compensation with significant upside potential and management possibilities. Resume to **e-mail: fmc4@spsfind.com.**

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**SENIOR POSTDOCTORAL FELLOWSHIP POSITION** is available at Northwestern University, to work on projects on the mechanisms of interferon signal transduction. Previous experience in molecular biology/biochemistry is required. Only senior Postdoctoral Fellows who have completed or are completing a first postdoctoral fellowship will be considered for this position. Applicants should submit electronically their curriculum vitae to the attention of: **Leonidas C. Platanias, M.D., Ph.D., Professor of Medicine, Deputy Director Robert H. Lurie Comprehensive Cancer Center, Northwestern University Medical School, Chicago, IL 60611. E-mail: cancercenteradmin@northwestern.edu.**

## POSITIONS OPEN

### UNIVERSITY OF MIAMI
**School of Medicine**

**POSTDOCTORAL POSITIONS** to study mechanisms of tissue growth regulation in the laboratories of **Leonidas Koniaris, M.D.** (liver and intestinal regeneration) and **Teresa Zimmers, Ph.D.** (skeletal muscle wasting) at the University of Miami/Sylvester Comprehensive Cancer Center. Qualified individuals must have a Ph.D. in a biomedical field and over five years of work experience. A strong background in molecular biology, cell biology, and pathology is required. Experience in rodent surgery is essential, including rat liver transplantation, murine 90 percent hepatectomy and 50 percent enterectomy. Excellent publication record and ability to initiate and complete independent research required. Salary $30,000 to $45,000, commensurate with experience.

Interested individuals should e-mail their curriculum vitae and contact information for three references to **Leonidas Koniaris, M.D., e-mail: lkoniaris@med.miami.edu** or **Teresa A. Zimmers, Ph. D., e-mail: tzimmers@med.miami.edu.**

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**INDIANA UNIVERSITY CENTER FOR DIABETES RESEARCH. A POSTDOCTORAL POSITION** will be available starting July 1, 2006, for a period of two years for training in the general area of diabetes and obesity. The position is supported by an NIH training grant and the salary will follow NIH guidelines. Applicants must choose a funded laboratory from among the faculty associated with the program (see **website: http://www.diabetes.iu.edu/**). Eligibility requires a Ph.D., M.D. or equivalent degree and *U.S. citizenship or permanent resident status.* Applicants should submit curriculum vitae and a statement of future goals along with the names of at least two references by June 1, 2006, to **Dr. Peter J. Roach** at **e-mail: proach@iupui.edu** or by mail to: **Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, 635 Barnhill Drive, M.S. 4053, Indianapolis, IN 46202-5122.** *Indiana University is an Equal Employment Opportunity/Affirmative Action Employer, Minorites/Women/Persons with Disabilities.*

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### POSTDOCTORAL RESEARCHER

The Division of Cardiothoracic Surgery at the Ohio State University Medical Center is seeking applications for Postdoctoral Researchers from candidates with Ph.D. degree to work on areas related to cardiovascular diseases. Strong on-hand experience in cell and molecular biological techniques required. Candidates with expertise in any of the following areas will be preferred: apoptosis, macrophage biology, lipoprotein receptors, and oxidative stress. Send application, curriculum vitae, and cover letter to **Dr. Parthasarathy, e-mail: spartha@osumc.edu.** *The Ohio State University is an Equal Opportunity/Affirmative Action Employer. Qualified women, minorities, Vietnam-era veterans, disabled veterans, and individuals with disabilities are encouraged to apply.*

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Several **POSTDOCTORAL RESEARCH POSITIONS** are available in the Ghosh laboratory at the University of Arizona **(website: http://www.chem.arizona.edu/)** to develop: (a) protein and peptide therapeutics as anti-Amyloid and anti-HIV agents and (b) DNA detection methodologies utilizing designed proteins. Applicants should have demonstrated expertise in peptide/protein chemistry with a strong publication record. Applications must be submitted at **website: http://www.hr.arizona.edu/.** (Job number 34752)

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### HARVARD MEDICAL SCHOOL

**POSTDOCTORAL POSITION** available to investigate the mechanisms of Alzheimer's and Parkinson's diseases through functional studies of disease genes in relevant neural circuits. Recent Ph.D.s with strong background in slice electrophysiology are encouraged to apply. Send curriculum vitae to: **Dr. Jie Shen (website: http://www.shenlab.net)** at **e-mail: jshen@rics.bwh.harvard.edu.**

## POSITIONS OPEN

### POSTDOCTORAL POSITION
**7T Small Animal Imagers**
**University of Nebraska Medical Center**

The Department of Radiology invites applications for a Postdoctoral position in MRI/MRSI of lithium in rodent models. The successful candidate must possess (or be graduating soon with) a Ph.D. in physics, biophysics, engineering, chemistry, neuroscience, or a related field with experience in programming and MRI data acquisition; postprocessing will be considered a plus. The successful candidate will engage in research on the delvelopment and application of MRSI of nonhydrogen nuclei for neuroradiology research. Submit curriculum vitae and names of three references to:

**S. Ramaprasad, Ph.D.**
**Associate Professor/ UNMC MR Physicist**
**Department of Radiology**
**981045 Nebraska Medical Center**
**Omaha NE 68198-1045**
**E-mail: sramaprasad@unmc.edu**

*The University of Nebraska is an Equal Opportunity/Affirmative Action Employer. Minorities/Women/Persons with Disabilities/Veterans.*

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**POSTDOCTORAL RESEARCH ASSOCIATE/GRADUATE RESEARCH ASSISTANT** positions are available in the Lung Biology and Toxicology Laboratory at Oklahoma State University (OSU) to study exocytosis, cell differentiation, ion channels, microRNA and/or fetal lung development from molecular level to whole animal using various modern techniques including DNA microarray and RNA interference. Highly motivated candidates are encouraged to apply. A background in molecular and cellular biology, electrophysiology, lung biology, and/or animal physiology is a plus. Send curriculum vitae to: **Dr. Lin Liu, Department of Physiological Sciences, Oklahoma State University, Stillwater, OK 74078-2014; e-mail: lin.liu@okstate.edu.** Applications will be accepted through May 31, 2006, or until suitable candidates are identified. *OSU is an Affirmative Action/Equal Opportunity Employer.*

## MARKETPLACE

# GE & Science Prize for Young Life Scientists

# Your essay may be the winner this year

GE & Science Prize for Young Life Scientists was established in 1995, and is presented by *Science*/AAAS and GE Healthcare. The prize was established to help bring science to life by recognizing outstanding PhDs from around the world and rewarding their research in the field of molecular biology.

This is your chance to gain international acclaim and recognition for yourself and your faculty, as well as to turn your scientific ideas into reality. If you were awarded your PhD in molecular biology* during 2005, describe your work in a 1,000-word essay. Then submit it for the 2006 GE & Science Prize for Young Life Scientists. Your essay will be reviewed by a panel of distinguished scientists who will select one grand prizewinner and four regional winners.

The grand prizewinner will get his or her essay published in *Science*, receive US$25,000, and be flown to the awards ceremony in Stockholm, Sweden. Entries should be received by **July 15, 2006**.

GE & Science Prize for Young Scientists: Life Science Re-imagined.

For more information on how to enter, go to www.gehealthcare.com/science

Established and presented by:

Science AAAS

* For the purpose of this prize, molecular biology is defined as "that part of biology which attempts to interpret biological events in terms of the physico-chemical properties of molecules in a cell" (McGraw-Hill Dictionary of Scientific and Technical Terms, 4th Edition).